10x GENOMICS

Sample Prep User Guide | CG000595 | Rev B

Reagent Assembly, Sample Labeling & Flow Sorting

For Barcode Enabled Antigen Mapping (BEAM)

For use with:

Chromium Single Cell 5' BEAM Core Kit, PE, Set A
128 rxns PN-1000539

Chromium Human MHC Class I A0201 Monomer Kit,
32 rxns PN-1000542

Chromium Human MHC Class I A1101 Monomer Kit,
32 rxns PN-1000543

Chromium Human MHC Class I B0702 Monomer Kit,
32 rxns PN-1000544

Chromium Human MHC Class I A2402 Monomer Kit,
32 rxns PN-1000545

Chromium Mouse MHC Class I H2Kb Monomer Kit,
32 rxns PN-1000546

Notices

Document Number

CG000595 | Rev B

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Document Revision Summary

Document Number

CG000595 | Rev B

Title

Reagent Assembly, Sample Labeling & Flow Sorting for Barcode Enabled Antigen Mapping (BEAM)

Revision

Rev B

Revision Date

April 2023

General Changes

Updated for general minor consistency of language and terms throughout

Specific Changes

- **Additional Kits, Reagents & Equipment Table**
  - » Added Biotinylated Human Serum Albumin and Mouse Serum Albumin (page 7)
- **Tips & Best Practices**
  - » Pre-enrichment of B & T Cells: Removed MACS Miltenyi B Cell Isolation Kit II and added EasySep Human B Cell Isolation Kit (STEMCELL Technologies PN-17954) (page 11)
  - » Added new section on Labeling & Wash Buffer (page 12)
  - » Added "Quenching should be performed" under BEAM-Ab Antigen Pre-screening (page 12)
  - » Updated guidance on the Negative Control BEAM-Ab Assembly (page 12)
- **Step 1: Pre-screen Antigens**
  - » Modified BEAM-Ab Pre-screening steps to include guidance on preparing Negative Control Assembly with Off-target Antigen (page 19 & 20)
- **Step 2: Test Multiple Antigens**
  - » Modified Step 2: Test Multiple Antigens to include guidance on preparing Negative Control Assembly with Off-target Antigen (page 23 & 24)
- **Troubleshooting**
  - » Added new guidance on background PE signal in cells labeled with the Negative Control BEAM-Ab Assembly (page 51)

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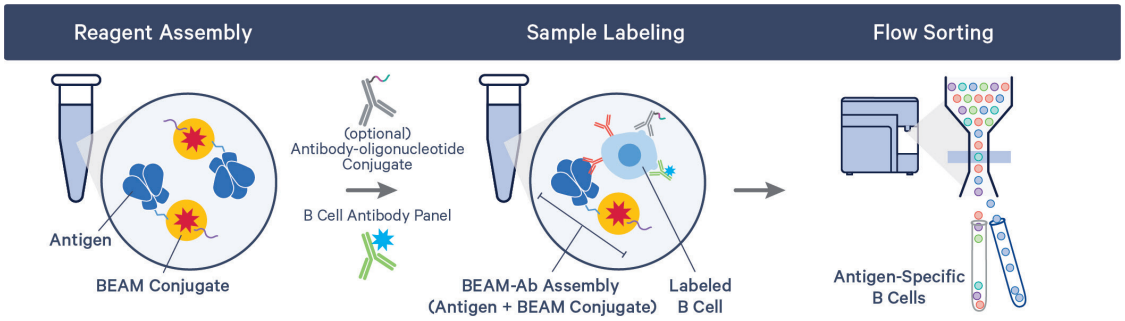
Objective

Chromium Single Cell 5' Barcode Enabled Antigen Mapping (BEAM) enables multiplexed screening of antigen targets to match unique antigens with their corresponding B-cell receptors (BCRs) and T-cell receptors (TCRs), allowing rapid discovery of antigen-specific BCRs (BEAM-Ab) and TCRs (BEAM-T), respectively.

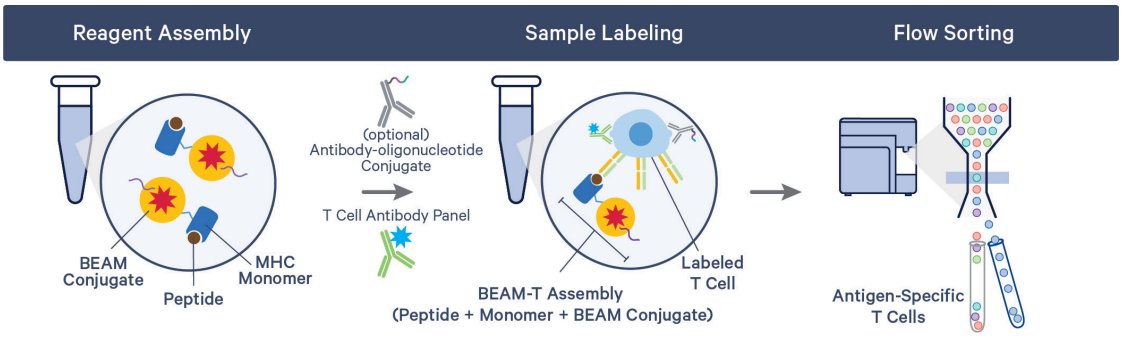
Before starting with the BEAM workflow, appropriate antigens and peptides must be obtained. Chromium Single Cell 5' BEAM Core Kit, PE, Set A 128 rxns PN-1000539 provides cell labeling and reagent assembly solution through the use of a set of 16 unique BEAM Conjugates, each composed of a streptavidin, a fluorophore molecule (Phycoerythrin, PE), and a Feature Barcode oligonucleotide. The antigens (for BEAM-Ab) or peptides (for BEAM-T) are first assembled with BEAM Conjugates to prepare BEAM-Ab or BEAM-T Assemblies, respectively. BEAM-T Assembly also requires MHC monomers (part of 10x Genomics Chromium Human/Mouse MHC Class I Monomer Kits). These assembled reagents are then used to label individual cells and the labeled cells are sorted through flow cytometry to collect antigen-specific B or T cells prior to loading onto a 10x Genomics chip.

This document provides guidance for preparing BEAM assemblies, cell labeling, and flow sorting for both BEAM-Ab and BEAM-T workflows. Cells prepared using this document can be used as input into the relevant Chromium Single Cell Immune Profiling Solutions User Guide with Feature Barcode technology (see References).

Barcode Enabled Antigen Mapping of Antigen-Specific BCRs (BEAM-Ab) – Sample Preparation Overview



Barcode Enabled Antigen Mapping of Antigen-Specific TCRs (BEAM-T) – Sample Preparation Overview



Additional Kits, Reagents & Equipment

The items in the tables below have been tested by 10x Genomics and perform optimally with the Chromium Single Cell 5' Barcode Enabled Antigen Mapping (BEAM) protocols. Substituting materials may adversely affect system performance. This list does not include standard laboratory equipment, such as water baths, pH meters, freezers, etc.

Item	Description		Supplier	Part Number (US)
Plastics				
0.2 ml PCR 8-tube strips	PCR Tubes 0.2 ml 8-tube strips	Choose either Eppendorf, USA Scientific or Thermo Fisher Scientific PCR 8-tube strips.	Eppendorf	951010022
	TempAssure PCR 8-tube strip		USA Scientific	1402-4700
	MicroAmp 8-Tube Strip, 0.2 ml MicroAmp 8 -Cap Strip, clear		Thermo Fisher Scientific Thermo Fisher Scientific	N8010580 N8010535
1.5-ml tubes	DNA LoBind Tubes 1.5 ml		Eppendorf	22431005
2-ml tubes	DNA LoBind Tubes 2.0 ml		Eppendorf	22431048
15-ml tubes	Corning 15 ml centrifuge tubes	Choose either tubes for reagent & buffer preparation.	Corning	CLS430791
50-ml tubes	Corning 50 ml centrifuge tubes		Corning	CLS430829
Kits & Reagents				
Sterile 1X PBS	Phosphate-Buffered Saline without Calcium & Magnesium *Or any equivalent sterile PBS*		Corning	21-040-CM
Nuclease-free water	Molecular Grade Nuclease-free Water		Thermo Fisher Scientific	AM9937
FBS	Fetal Bovine Serum, qualified, heat inactivated		Thermo Fisher Scientific	16140071
	Avantor Seradigm Premium Grade Fetal Bovine Serum		VWR	97068-085
FcX	Human TruLabel FcX (Fc Receptor Blocking Solution) TruStain FcX™ PLUS (anti-mouse CD16/32) Antibody		Biolegend Biolegend	422301 156603
Total-Seq C	TotalSeq™ Antibody Oligonucleotide Conjugates *Optional*		Biolegend	-
Antibodies	See Example Antibody Panels		-	-
Viability dye	Invitrogen eBioscience 7-AAD Viability Staining Solution		Invitrogen	00699350
BSA	UltraPure Bovine Serum Albumin (BSA, 50 mg/ml) MACS BSA Stock Solution		Thermo Fisher Scientific Miltenyi Biotec	AM2616 130-091-376
Biotinylated HSA	Biotinylated human serum albumin *Optional*		Acro Biosystems	HSA-H82E3
Biotinylated MSA	Biotinylated mouse serum albumin *Optional*		Acro Biosystems	MSA-M82E4

Cell Counting				
Label	Trypan Blue Label (0.4%)	Choose counter based on availability & preference.	Thermo Fisher Scientific	T10282
Cell counter & slides	Countess II FL Automated Cell Counter Countess 3 FL Automated Cell Counter Countess Cell Counting Chamber Slides		Thermo Fisher Scientific Thermo Fisher Scientific Thermo Fisher Scientific	AMQAF1000 A49866 C10228
Equipment				
Vortex	Vortex Mixer		VWR	10153-838
Centrifuge	Refrigerated Eppendorf Centrifuge *Or any equivalent centrifuge*		Millipore-Sigma	5427R or 5424R
Sorter	MA900 Multi-Application Cell Sorter *Or any equivalent cell sorter*		Sony	MA900

For some items, a number of vendor options are listed. Choose item based on availability and preference. Refer to the manufacturer's website for regional part numbers.

Recommended Pipette Tips

10x Genomics recommends using only validated emulsion-safe pipette tips for all Single Cell protocols. Rainin pipette tips have been extensively validated by 10x Genomics and are highly recommended for all single cell assays. If Rainin tips are unavailable, any of the listed alternate pipette tips validated by 10x Genomics may be used.

Supplier	Description	Part Number (US)
Recommended Pipettes & Pipette tips		
Rainin (pipettes)	Pipet-Lite Multi Pipette L8-50XLS+	17013804
	Pipet-Lite Multi Pipette L8-200XLS+	17013805
	Pipet-Lite Multi Pipette L8-10XLS+	17013802
	Pipet-Lite Multi Pipette L8-20XLS+	17013803
	Pipet-Lite LTS Pipette L-2XLS+	17014393
	Pipet-Lite LTS Pipette L-10XLS+	17014388
	Pipet-Lite LTS Pipette L-20XLS+	17014392
	Pipet-Lite LTS Pipette L-100XLS+	17014384
	Pipet-Lite LTS Pipette L-200XLS+	17014391
	Pipet-Lite LTS Pipette L-1000XLS+	17014382
Rainin (pipette tips)	Tips LTS 200UL Filter RT-L200FLR	30389240
	Tips LTS 1ML Filter RT-L1000FLR	30389213
	Tips LTS 20UL Filter RT-L10FLR	30389226
Alternate Recommendations *(If Rainin pipette tips are unavailable, any of the listed pipette tips may be used)*		
Eppendorf (pipettes)	Eppendorf Research plus, 8-channel, epT.I.P.S. Box, 0.5 – 10 µL	3125000010
	Eppendorf Research plus, 8-channel, epT.I.P.S. Box, 10 – 100 µL	3125000036
	Eppendorf Research plus, 8-channel, epT.I.P.S. Box, 100 – 300 µL	3125000052
	Eppendorf Research plus, 1-channel, epT.I.P.S.® Box, 0.1 – 2.5 µL	3123000012
	Eppendorf Research plus, 1-channel, epT.I.P.S.® Box, 0.5 – 10 µL	3123000020
	Eppendorf Research plus, 1-channel, epT.I.P.S.® Box, 2 – 20 µL	3123000039
	Eppendorf Research plus, 1-channel, epT.I.P.S.® Box, 2 – 200 µL	3123000055
	Eppendorf Research plus, 1-channel, epT.I.P.S.® Box, 100 – 1000 µL	3123000063
Eppendorf (pipette tips) *Compatible with Eppendorf pipettes only*	ep Dualfilter T.I.P.S., 2-20 µL	0030078535
	ep Dualfilter T.I.P.S., 2-200 µL	0030078551
	ep Dualfilter T.I.P.S., 2-1,000 µL	0030078578
Labcon*	ZAP SLIK 20 µL Low Retention Aerosol Filter Pipet Tips for Rainin LTS	4-1143-965-008
	ZAP SLIK 200 µL Low Retention Aerosol Filter Pipet Tips for Rainin LTS	4-1144-965-008
	ZAP SLIK 1000 µL Low Retention Aerosol Filter Pipet Tips for Rainin LTS	4-1145-965-008
Biotix*	xTIP4 Racked Pipette Tips, Rainin LTS Pipette Compatible, 0.1-20uL	63300931
	xTIP4 Racked Pipette Tips, Rainin LTS Pipette Compatible, 200uL	63300001
	xTIP4 Racked Pipette Tips, Rainin LTS Pipette Compatible, 1000uL	63300003

**Compatible with Rainin pipettes*

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Tips & Best Practices

Icons

TIPS		BEAM-Ab	BEAM-T
Tips & Best Practices section includes additional guidance	Signifies critical step requiring accurate execution	Guidance/steps for Barcode Enabled Antigen Mapping of antigen-specific BCRs (BEAM-Ab)	Guidance/steps for Barcode Enabled Antigen Mapping of antigen-specific TCRs (BEAM-T)

Plastics

- Use recommended plastic consumables when performing reagent assembly and labeling as some plastics can introduce fibers into reagents, buffers, and solutions, leading to microfluidic failures.

General Reagent Handling

- Fully thaw and thoroughly pipette mix reagents before use.
- Keep all reagents on ice during setup and use. Promptly move reagents back to the recommended storage after use.
- BEAM Conjugates are light sensitive. When transferring them into clear tubes for assembly, cover the tubes with an aluminum foil and keep in the dark.

Pipette Tips & Calibration

- Follow manufacturer's calibration and maintenance schedules.
- Use only recommended pipette tips.

Centrifugation Conditions

- Centrifugation speed and time depends upon the sample type.
- Larger or fragile cell types may require slower centrifugation speeds.
- Use of swinging-bucket rotor is recommended for higher cell recovery.
- Post-sorting, cells should be centrifuged at **150 rcf** for **10 min** at **4°C**, if needed.

Pre-enrichment of B and T Cells

- If the percentage of T or B cells in the starting sample is low, it is recommended to enrich the sample for the cell type of interest (B or T cells) to maximize the use of BEAM Conjugate and to decrease flow sorting time.
- Use a negative selection kit for pre-enrichment to avoid interference with BEAM reagents.
- Examples of some negative selection kits include Dynabeads Untouched Human CD8 T Cells Kit (Invitrogen PN-11348D), Dynabeads Untouched Mouse CD8 T Cells Kit (Invitrogen PN-11417D), or EasySep Human B Cell Isolation Kit (STEMCELL Technologies PN-17954).

Sample Types & Viability

- This protocol has been demonstrated on human PBMCs, enriched T and B cells from human PBMCs, mouse splenocytes, and mouse lymphocytes.
- High viability samples give best results. Use samples with at least 70% viability for Barcode Enabled Antigen Mapping (BEAM) workflow. After sorting, the viability should be >90%.

Labeling & Wash Buffer

- Use chilled PBS + 2% FBS for labeling and washing.
- DO NOT use alternative buffers.
- BSA-containing buffers may increase background.

BEAM-Ab Assembly

BEAM-Ab

- BEAM-Ab Assembly preparation involves combining a biotinylated antigen with a unique BEAM Conjugate.
- One Negative Control Assembly should also be set up per sample.
- BEAM-Ab Assemblies should also be quenched before pooling and cell labeling.

Antigen Sourcing & Pre-screening

BEAM-Ab

- Use a high-quality biotinylated antigen for BEAM-Ab assembly.
- Note the molecular weight and concentration of each antigen before proceeding with the assembly.
- Up to 15 different target antigens (and one negative control) can be tested using the BEAM-Ab workflow.
- Antigen can vary in size from 10 - 200 kDa. Consult Barcode Enabled Antigen Mapping (BEAM) Experimental Planning Guide (Document CG000596) for further details on antigen specifications.

Antigen Pre-screening

BEAM-Ab

- Pre-screen each biotinylated antigen individually before starting reagent assembly to determine the antigen quality.
- During antigen pre-screening:
  - » A non-experimental sample can be used.
  - » Quenching should be performed.
  - » DO NOT pool the assemblies.
  - » Label sample with a unique assembly.
  - » Once labeled, proceed to flow cytometry analysis without sorting.

Negative Control Assembly

BEAM-Ab

- The Negative Control BEAM-Ab assembly can be prepared using a biotinylated off-target antigen (human/mouse serum albumin) or using 1X PBS in the place of the antigen.
- If bead-based pre-enrichment will be performed, using a biotinylated off-target antigen for the Negative Control Assembly is recommended, as it may reduce background.

BEAM-T Assembly

BEAM-T

- BEAM-T Assembly preparation involves combining a peptide, an appropriate MHC-matched monomer and a unique BEAM Conjugate.
- BEAM-T Assemblies should also be quenched before pooling.
- One Negative Control Peptide Assembly should also be set up per monomer.

MHC Monomer & Target Peptide

BEAM-T

- Each Chromium Human/Mouse MHC Class I Kit includes a biotinylated MHC monomer.
- Use appropriate, high-quality MHC monomer-matched peptide.
- Peptide should be 9-12 amino acids long. Consult Barcode Enabled Antigen Mapping (BEAM) Experimental Planning Guide (Document CG000596) for further details on peptide specifications.
- Up to 15 different target peptides (and one Negative Control Peptide) can be tested using the BEAM-T workflow.
- During assembly, use the appropriate matched monomer.
- More than one monomer can be used in one experiment. In this case, a separate negative control assembly would be required for each monomer.

Negative Control Peptide

BEAM-T

- Each Chromium Human/Mouse MHC Class I Kit includes a monomer-matched Negative Control Peptide.
- It is used to set up a Negative Control Peptide Assembly by combining Negative Control Peptide, appropriate MHC monomer, and unique BEAM Conjugate. Negative control assembly is required for downstream antigen specificity calculations in Cell Ranger.
- The Negative Control Peptide is also added to all the assemblies during quenching to ensure any improperly loaded MHC monomers are loaded before multiplexing.
- Use the appropriate monomer-matched Negative Control Peptide for Negative Control Assembly and for quenching.

Peptide Pre-screening

BEAM-T

- Pre-screen each peptide individually before proceeding with testing multiple peptides to determine the peptide quality and proper loading of the MHC monomer.
- During peptide pre-screening:
  - » A non-experimental sample can be used.
  - » Quenching is not needed.
  - » DO NOT pool the assemblies.
  - » Label sample with a unique assembly.
  - » Once labeled, proceed to flow cytometry analysis without sorting.

Antibody Panels for Flow Sorting

BEAM-Ab

BEAM-T

- Marker panels for flow sorting should contain the following components:
  - » **Markers for Desired Cells**: Antibodies specifically labeling T cells (BEAM-T) or B cells (BEAM-Ab) should be included.
  - » **Markers for Undesired Cell Lineages**: Additionally, markers for cells that are not desired should also be included so that these cell populations can be excluded during sorting.
  - » **Live Dead Marker**: These markers selectively label dead cells, allowing the sorter to distinguish between live and dead cell populations. They function by entering dead cells (whose cell membranes are compromised) and binding to DNA or free amine groups. The Barcode Enabled Antigen Mapping assay was validated with 7-AAD.
- Phycoerythrin (PE) is the fluorophore for enriching BEAM^{+} antigen-specific cells, so the antibodies must be selected to work with or compensated appropriately to work with PE.
- Examples of human and mouse antibody panels for flow sorting are provided in the Appendix.
- Consult the Technical Note Barcode Enabled Antigen Mapping (BEAM) Flow Cytometry Guidelines (Document CG000598) for further guidance on antibody panels.
- Same antibody panels can be used for flow cytomtery when pre-screening antigens and peptides.
- Use dim fluorophores with abundant markers and use bright fluorophores with dim markers. Additional antibodies can be added as long as they do not interfere with PE.
- Use a PE based antibody to build compensation controls.

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Barcode Enabled Antigen Mapping of Antigen-Specific BCRs (BEAM-Ab) Protocol:

Assembly, Labeling & Flow Sorting

Reagent Kits

For use with the Chromium Next GEM Single Cell 5' Gene Expression with Feature Barcode technology for Barcode Enabled Antigen Mapping (BEAM)

Refer to SDS for handling and disposal information

Chromium Single Cell 5' BEAM Core Kit, PE, Set A 128 rxns PN-1000539

Chromium
Single Cell 5' BEAM Core Kit, PE, Set A
128 rxns, Module 1
PN-1000539
Store at -20°C

	#	PN
BEAM Conjugate 1, PE	1	2000774
BEAM Conjugate 2, PE	1	2000775
BEAM Conjugate 3, PE	1	2000776
BEAM Conjugate 4, PE	1	2000777
BEAM Conjugate 5, PE	1	2000778
BEAM Conjugate 6, PE	1	2000779
BEAM Conjugate 7, PE	1	2000780
BEAM Conjugate 8, PE	1	2000781
Quenching Reagent	1	2000790

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Chromium
Single Cell 5' BEAM Core Kit, PE, Set A
128 rxns, Module 2
PN-1000539
Store at -20°C

	#	PN
BEAM Conjugate 9, PE	1	2000782
BEAM Conjugate 10, PE	1	2000783
BEAM Conjugate 11, PE	1	2000784
BEAM Conjugate 12, PE	1	2000785
BEAM Conjugate 13, PE	1	2000786
BEAM Conjugate 14, PE	1	2000787
BEAM Conjugate 15, PE	1	2000788
BEAM Conjugate 16, PE	1	2000789
Quenching Reagent	1	2000790

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Cap colors for BEAM Conjugates 1-16 tubes may vary depending on the lot. Verify reagent using the part number.

Protocol Steps & Timing

The table below provides an overview of the Barcode Enabled Antigen Mapping of antigen-specific BCRs (BEAM-Ab) protocol steps and timing.

	Steps	Timing	Stop & Store
Day 1	**Step 1. Pre-screen Antigens***		
	(Includes preparing BEAM-Ab Assembly, sample labeling, and flow cytometric analysis)	2-3 h	
Day 2	**Step 2. Test Multiple Antigens**		
	Step 2.1. BEAM-Ab Assembly Preparation	~1 h STOP	4°C ≤24 h
	Step 2.2. Sample Labeling	~1 h	
	Step 2.3. Flow Sorting	2-3 h	

**Antigen pre-screening is required to determine the antigen quality and therefore multiplexing compatibility. This step can be skipped if the antigen is already pre-screened.*

*Keep samples on ice following sorting. Proceed **immediately** to the Chromium Single Cell Immune Profiling Solutions User Guide with Feature Barcode technology (see References).*

Get Started

Cap colors for BEAM Conjugates 1-16 tubes may vary depending on the lot. Verify reagent using the part number.

	Item	10x PN	Preparation & Handling	Storage
	Place on Ice			
●	**BEAM Conjugate 1-16, PE**	2000774 - 2000789	Thaw at room temperature. Vortex, centrifuge briefly, pipette mix 5x (pipette set at 20 µl), and place on ice.	–20°C
○	**Quenching Reagent**	2000790	Thaw at room temperature. Vortex, verify no precipitate, and centrifuge briefly. Place on ice.	–20°C
	Biotinylated Antigen *Use appropriate, high-quality biotinylated antigen in the size range of 10 - 200 KDa.*	—	Note the stock concentration and molecular weight of the antigen. Manufacturer's recommendations.	—
	Biotinylated Human/ Mouse Serum Albumin *Optional*	—	Note the stock concentration and molecular weight of the antigen. Manufacturer's recommendations.	—
	BEAM-Ab Antibody Panel for Flow Sorting *See Appendix for examples.*	—	Manufacturer's recommendations.	—
	7-AAD (7-Aminoactinomycin D)	—	Manufacturer's recommendations.	4°C
	Fc Receptor Blocking Solution Human/Mouse	—	Manufacturer's recommendations.	—
	TotalSeq™-C Antibody Oligonucleotide Conjugates *Optional For simultaneous cell surface protein labeling*	—	Manufacturer's recommendations.	—
	Obtain			
	Sterile 1X PBS	—	—	4°C
	BSA *Optional For preparing PBS + 1% BSA for resuspending lyophillized antibody-oligonucleotide conjugates*	—	—	4°C
	Vortex	—	—	—
	FBS	—	—	4°C

Step 1: Pre-screen Antigens

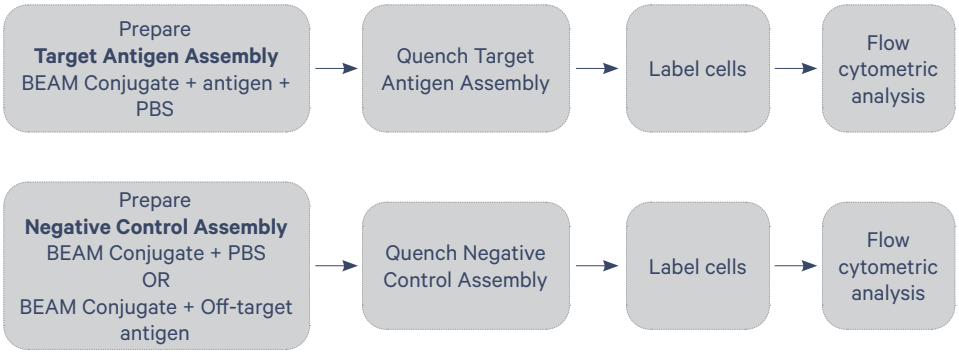
Pre-screen each antigen before starting BEAM-Ab workflow to determine antigen quality. Poor quality antigen can alter the PE signal during flow sorting. This step only needs to be performed once. For antigens already screened, proceed directly to step 2. Pre-screening does not require a positive signal and thus can be performed on non-experimental samples.

For the purpose of pre-screening the antigens, the following two unique BEAM-Ab Assemblies should be prepared:

i. Target Antigen Assembly

ii. Negative Control Assembly

Pre-screening Overview



BEAM-Ab Assembly for Pre-screening

a. Record the stock concentration and molecular weight of each antigen. Use the Barcode Enabled Antigen Mapping (BEAM) Workbook for BEAM-Ab Assembly (Document CG000597) to determine the volume of the antigen required for each assembly.

b. Target Antigen Assembly
Add reagents in the order listed. Pipette mix and keep on ice in the dark.

BEAM Assembly	PN	For 1 BEAM-Ab Assembly (µl)
Sterile 1X PBS	-	Variable
● **BEAM Conjugate 1-16, PE**	2000774 - 2000789	2.0
Biotinylated Antigen	-	Variable*
Total	**-**	**40.0**

Record the BEAM Conjugate used to assemble each antigen.

**The volume of antigen required is dependent on the antigen stock concentration and molecular weight. This volume can be calculated using the Barcode Enabled Antigen Mapping (BEAM) Workbook for BEAM-Ab Assembly (Document CG000597). Add an appropriate volume of PBS based on the amount of added antigen to achieve the stated total volume.*

Select either step c or d for Negative Control Assembly. If bead-based pre-enrichment was performed, step d is recommended as it may reduce background. See Troubleshooting section for further details.

c. Negative Control Assembly with No Antigen

Add reagents in the order listed. Pipette mix and keep on ice in dark. Use a unique BEAM Conjugate for Negative Control Assembly.

BEAM Assembly	PN	For 1 BEAM-Ab Assembly (µl)
Sterile 1X PBS	-	38.0
BEAM Conjugate 1-16, PE	2000774 - 2000789	2.0
Total	**-**	**40.0**

d. Negative Control Assembly with Off-Target Antigen

Add reagents in the order listed. Pipette mix and keep on ice in dark. Use a unique BEAM Conjugate for Negative Control Assembly.

BEAM Assembly	PN	For 1 BEAM-Ab Assembly (µl)
Sterile 1X PBS	-	Variable
BEAM Conjugate 1-16, PE	2000774 - 2000789	2.0
Biotinylated Human/ Mouse Serum Albumin	-	Variable*
Total	**-**	**40.0**

**The volume of antigen required is dependent on the antigen stock concentration and molecular weight. This volume can be calculated using the Barcode Enabled Antigen Mapping (BEAM) Workbook for BEAM-Ab Assembly (Document CG000597). Add an appropriate volume of PBS based on the amount of added antigen to achieve the stated total volume.*

e. Incubate the assemblies for **30 min** on **ice** in the dark.

f. Add **5 µl** Quenching Reagent to each assembly and pipette mix 5x.

STOP

g. Incubate for **30 min** on **ice** in the dark or store the BEAM-Ab Assemblies for up to **24 h** at **4°C** in the dark.

h. To minimize bubble formation, centrifuge the assemblies at **2,500 rcf** for **5 min** at **4°C**.

i. Maintain the assembly at **4°C** in the dark and proceed **immediately** to the next step.

Sample Labeling

This protocol was demonstrated using 0.2-1 x 10^6 cells. Wash and thaw cells according to the appropriate 10x Genomics Demonstrated Protocol for the cell type being prepared.

a. Prepare PBS + 2% FBS and keep on ice.

b. Prepare B cell antibody panel for flow cytometry at appropriate dilution and keep on ice in the dark. See Appendix for examples panels for human and mouse.

c. Dispense 0.2-1 x 10^6 cells each into a new 15-ml centrifuge tube for each Target Antigen Assembly or Negative Control Assembly.

d. Centrifuge cells at **300 rcf** for **5 min** at **4°C**.

e. Remove the supernatant.

f. Resuspend each pellet in **90 µl** chilled PBS + 2% FBS and keep on ice.

g. Add **10 µl** human/mouse Fc Receptor Blocking Solution, pipette mix, and incubate for **10 min** on ice.

h. Add appropriate volume of B cell antibody panel for flow cytometry to the cells and pipette mix. The volume will depend on the number of cells.

i. Add **7.2 µl** of appropriate BEAM-Ab Assembly to the cells in each tube. Using a P1000 pipette, gently mix 5x.

j. Incubate for **30 min** on **ice** in the dark and gently pipette mix cells every **10 min**.

k. Add **3.5** ml chilled PBS + 2 % FBS. Gently pipette mix 5x.

l. Centrifuge at **300 rcf** for **5 min** at **4°C**.

m. Remove the supernatant.

n. **Repeat** k - m 2x for a total of three washes.

o. Resuspend cells in **100 µl** chilled PBS + 2% FBS. Pipette mix 5x.

p. Add **0.5 µl** 7-AAD per **100 µl** sample.

q. Maintain samples on ice in the dark and proceed to flow cytometry.

Flow Cytometry

a. Prepare appropriate compensation controls. Compensation controls should be prepared fresh for every experiment. For further details, consult Technical Note Barcode Enabled Antigen Mapping (BEAM) Flow Cytometry Guidelines (Document CG000598).

b. Prepare fluorescence minus one (FMO) controls. See Appendix for details.

c. Gate on lymphocytes by size (scatter), single cells, live cells (e.g. 7-AAD negative), lineage negative, $CD19^+$ cells, and then analyze dual positive PE^+ $CD19^+$ cells.

d. Analyze the results and proceed with step 2 if the antigen passes the pre-screening. See Appendix for guidance on Antigen Pre-screening Analysis for BEAM-Ab.

Step 2: Test Multiple Antigens

Step 2.1: BEAM-Ab Assembly Preparation

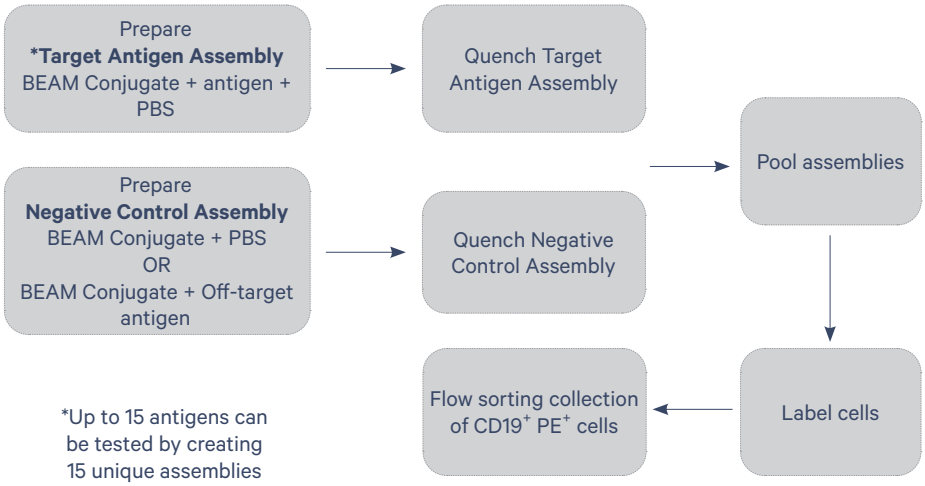
This section provides guidance on testing multiple antigens in a sample. A downloadable workbook (Barcode Enabled Antigen Mapping (BEAM) Workbook for BEAM-Ab Assembly, Document CG000597) for the calculations described in this section is also available on the 10x Genomics Support website and can be used concurrently with this user guide.

For the purpose of testing multiple antigens, the following two types of BEAM-Ab Assemblies should be prepared:

- One or more Target Antigen Assemblies
- Negative Control Assembly

A Negative Control Assembly is critical for calculating antigen specificity scores.

Testing Multiple Antigens Overview



a. Record the stock concentration and molecular weight of each antigen. Use the Barcode Enabled Antigen Mapping (BEAM) Workbook for BEAM-Ab Assembly (Document CG000597) to determine the volume of the antigen required for each assembly.

b. Target Antigen Assembly
Add reagents in the order listed. Pipette mix and maintain on ice in the dark.

BEAM Assembly	PN	For 1 BEAM-Ab Assembly (µl)
Sterile 1X PBS	-	Variable
● **BEAM Conjugate 1-16**	2000774 - 2000789	2.0
Biotinylated Antigen	-	Variable*
Total	**-**	**40.0**

Record the BEAM Conjugate used to assemble each antigen.

**This volume can be calculated using the Barcode Enabled Antigen Mapping (BEAM) Workbook for BEAM-Ab Assembly (Document CG000597). Add an appropriate volume of PBS based on the amount of added antigen to achieve the stated total volume.*

Select either step c or d for Negative Control Assembly. If bead-based pre-enrichment was performed, step d is recommended as it may reduce background. See Troubleshooting section for further details.

c. Negative Control Assembly with No Antigen

Add reagents in the order listed. Pipette mix and maintain on ice in the dark. Use a unique BEAM Conjugate for negative control assembly.

Record the BEAM Conjugate used to prepare the Negative Control Assembly.

BEAM Assembly	PN	For 1 BEAM-Ab Assembly (µl)
Sterile 1X PBS	-	38.0
BEAM Conjugate 1-16	2000774 - 2000789	2.0
Total	**-**	**40.0**

d. Negative Control Assembly with Off-target Antigen

Add reagents in the order listed. Pipette mix and maintain on ice in the dark. Use a unique BEAM Conjugate for negative control assembly.

BEAM Assembly	PN	For 1 BEAM-Ab Assembly (µl)
Sterile 1X PBS	-	Variable
BEAM Conjugate 1-16	2000774 - 2000789	2.0
Biotinylated Human/ Mouse Serum Albumin	-	Variable*
Total	**-**	**40.0**

**This volume can be calculated using the Barcode Enabled Antigen Mapping (BEAM) Workbook for BEAM-Ab Assembly (Document CG000597). Add an appropriate volume of PBS based on the amount of added antigen to achieve the stated total volume.*

e. Incubate the assemblies for **30 min** on **ice** in the dark.

f. Add **5 µl** Quenching Reagent to each assembly and pipette mix 5x.

STOP

g. Incubate for **30 min** on **ice** in the dark or store the BEAM-Ab Assemblies for up to **24 h** at **4°C** in the dark.

h. To minimize bubble formation, centrifuge the assemblies at **2,500 rcf** for **5 min** at **4°C** and keep the assemblies (**45 µl**) on ice in the dark.

i. Pool each Target Antigen Assembly and Negative Control Assembly in a tube. Pipette mix and maintain on the ice in the dark.
The volume of each BEAM-Ab Assembly for pooling will depend on the number of cells to be labeled in step 2.2. See the table below for guidance on appropriate volume to pool.

Number of Cells to be labeled in step 2.2	Total volume to prepare of each quenched BEAM-Ab Assembly (steps 2.1a-2.1g) (µl)	Volume of each BEAM-Ab Assembly for Pooling (step 2.1h) (µl)
200,000 - 1 x 10^6	45	8
1 x 10^6 - 2 x 10^6		16
2 x 10^6 - 3 x 10^6		24
3 x 10^6 - 4 x 10^6		32
4 x 10^6 - 5 x 10^6		40
5 x 10^6 - 6 x 10^6	90	48
6 x 10^6 - 7 x 10^6		56
7 x 10^6 - 8 x 10^6		64

8 µl of the final quenched assembly is needed to label up to 1 x 10^6 cells and thus the 45 µl volume prepared at step 2.1h can be used to label up to 5 x 10^6 cells, if needed. If working with more than 5 x 10^6 cells, scale up the volume of each BEAM-Ab Assembly (see table above).

Step 2.2: Sample Labeling

This protocol was demonstrated using 0.2-8 x 10^6 cells. For more than 8 x 10^6 cells, set up additional labeling reactions. Wash and thaw cells according to the appropriate 10x Genomics Demonstrated Protocol available on the 10x Genomics Support website.

a. Prepare PBS + 2% FBS and keep on ice. This buffer can be prepared a day before the experiment.

b. Prepare B cell antibody panel for flow sorting and keep on ice in the dark. See Appendix for some example panels.

c. **Optional Step** Prepare Antibody Mix Supernatant for optional cell surface protein labeling and keep on ice.

Prepare	
Antibody Mix Supernatant (containing antibody-oligonucleotide conjugates) *For optional cell surface protein labeling*	• Add appropriate/manufacturer's recommended amount of antibody-oligonucleotide conjugates to a 1.5-ml microcentrifuge tube. • If using a custom lyophillized antibody: Resuspend the antibody-oligonucleotide conjugates in an appropriate volume of PBS + 1% BSA. • Centrifuge the mix at 14,000 rcf for 10 min at 4°C. • Transfer the supernatant (containing Antibody Mix) to a new tube and maintain at 4°C.

d. Dispense 0.2-8 x 10^6 cells into a new 15-ml centrifuge tube.

e. Centrifuge at **300 rcf** for **5 min** at **4°C**.

f. Remove the supernatant.

g. Resuspend pellet in **90 µl** chilled PBS + 2% FBS and keep on ice.

h. Add appropriate volume of human/mouse Fc Receptor Blocking Solution (10 µl for up to 1 x 10^6 cells), pipette mix and incubate for **10 min** on **ice**. If working with more than 1 x 10^6 cells, scale up the volume of blocking solution accordingly.

i. Add appropriate volume B cell antibody panel for flow sorting and optional Antibody Mix Supernatant to the cells.

j. Add appropriate volume (7.2 µl per assembly per 1 x 10^6 cells) of pooled assembly from step 2.1i to the cells. For example, if the pool contains one Target Antigen Assembly and one Negative Control Assembly, 7.2 x 2 = 14.4 µl of pooled assemblies should be added. Using a P1000 pipette, gently mix 5x.

The volume of the pooled assembly added will depend on the number of cells to be labeled. If working with more than 1 x 10^6 cells, scale up the volume as needed. For example, if the pool contains one Target Antigen Assembly and one Negative Control Assembly and if 2 x 10^6 cells will be labeled, 7.2 x 4 = 28.8 µl of pooled assemblies should be added. The volume of assembly should be scaled up for every 1 x 10^6 total cells, regardless of the expected percentage of B cells in the sample.

TIPS

The Barcode Enabled Antigen Mapping (BEAM) Workbook for BEAM-Ab Assembly (Document CG000597) can also be used to calculate appropriate volumes for pooling.

k. Incubate for **30 min** on **ice** in the dark. Gently pipette mix every 10 min.

l. Add **3.5 ml** chilled PBS + 2 % FBS. Gently pipette mix 5x.

m. Centrifuge at **300 rcf** for **5 min** at **4°C**. Centrifugation speed and time depends upon the sample type.

n. Remove the supernatant without disturbing the pellet.

o. **Repeat** l-n 2x for a total three washes.

p. Resuspend cells in appropriate volume chilled PBS + 2% FBS. Pipette mix 5x.
Resuspension volume depends on the number of the cells. Resuspend in 100 µl for every 1 x 10^6 cells labeled. For example, for 8 x 10^6 labeled cells, resuspend pellet in 800 µl chilled PBS + 2% FBS.

q. Add **1.0 µl** 7-AAD per **200 µl** sample.

r. Proceed **immediately** to flow sorting with appropriate settings.

Step 2.3: Flow Sorting

a. Prepare PBS + 20% FBS for pre-coating the collection tubes.

b. Prepare Collection Buffer for sorted cells and maintain the buffer on ice. See the table below for guidance on choosing the appropriate Collection Buffer. This table also provides guidance on the appropriate volume for collection and post-sorting steps.

Collection Buffers and Post-Sorting Guidance

Collection Buffer	Volume for # cells sorted	Expected cell conc. (Confirm by counting)	Centrifugation to concentrate cells post-sorting?	Post-sorting notes
PBS + 5% FBS	20 µl for ≤5,000 cells	≤147 cells/µl	No	This volume can be accommodated in 1 chip well
PBS + 5% FBS	20 µl for ≤10,000 cells	≤208 cells/µl	No	This volume must be loaded in 2 chip wells
PBS + 20% FBS	20 µl for ≤20,000 cells	≤263 cells/µl	No	This volume must be loaded in 2 chip wells
PBS + 20% FBS	30 µl for 20,001-50,000 cells	232-294 cells/µl	No	This volume must be loaded in 4 chip wells
PBS + 20% FBS	30 µl for 50,001-500,000 cells	–	Recommended	• After sorting, add additional cold PBS+20% FBS for a total 1.5 ml volume. • Centrifuge at 150 rcf for 10 min at 4°C. • Count
100% FBS	30 µl for more than 500,000 sorted cells	–	Yes	• After sorting, add additional cold PBS+20% FBS for a total 1.5 ml volume. • Centrifuge at 150 rcf for 10 min at 4°C. • Count

When working with small numbers of cells (e.g., less than 50,000), expect poor (<75%) recovery of cells by centrifugation.

c. Pre-coat sorting collection tubes by adding **1 ml** PBS + 20% FBS to a tube then removing all of the PBS + 20% FBS.

d. Add an appropriate Collection Buffer (see the table above) to the collection tube.

e. Prepare appropriate compensation controls. Compensation controls should be prepared fresh for every flow sorting experiment. For further details, consult the Technical Note Barcode Enabled Antigen Mapping (BEAM) Flow Cytometry Guidelines (Document CG000598).

f. Prepare fluorescence minus one (FMO) controls. See Appendix for details.

g. Gate on lymphocytes by size (scatter), single cells, live cells (e.g. 7-AAD negative), lineage negative, $CD19^+$ cells and then sorting dual positive PE^+ $CD19^+$ cells.

h. Cells should be sorted using the purity mode on a low pressure setting (e.g. 100 µM nozzle, etc). Keep cells on ice and in the dark prior to sorting. During sorting, the collection tube should be chilled. Place the sorted cells immediately on ice after sorting.

Consult the Technical Note Barcode Enabled Antigen Mapping (BEAM) Flow Cytometry Guidelines (Document CG000598) for details on gating strategy and other cell sorting best practices.

i. Determine cell concentration and viability using an automated cell counter or a hemocytometer. Cell counting can be skipped if the sorted cell numbers are expected to be low.

If necessary, the collected cells may be concentrated by centrifugation at 150 rcf for 10 min at 4°C and by removing the supernatant (see Collection Buffers and Post-Sorting Guidance table). Use of a longer centrifugation time is recommended post sorting.

j. Proceed **immediately** to the relevant Chromium Single Cell Immune Profiling Solutions User Guide with Feature Barcode technology (see References).

10x GENOMICS

Barcode Enabled Antigen Mapping of Antigen-Specific TCRs (BEAM-T) Protocol:

Assembly, Labeling & Flow Sorting

Reagent Kits

For use with the Chromium Next GEM Single Cell 5' Gene Expression with Feature Barcode technology for Barcode Enabled Antigen Mapping (BEAM)

Refer to SDS for handling and disposal information

Chromium Single Cell 5' BEAM Core Kit, PE, Set A 128 rxns PN-1000539

Chromium
Single Cell 5' BEAM Core Kit, PE, Set A
128 rxns, Module 1
PN-1000539
Store at -20°C

	#	PN
BEAM Conjugate 1, PE	1	2000774
BEAM Conjugate 2, PE	1	2000775
BEAM Conjugate 3, PE	1	2000776
BEAM Conjugate 4, PE	1	2000777
BEAM Conjugate 5, PE	1	2000778
BEAM Conjugate 6, PE	1	2000779
BEAM Conjugate 7, PE	1	2000780
BEAM Conjugate 8, PE	1	2000781
Quenching Reagent	1	2000790

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Chromium
Single Cell 5' BEAM Core Kit, PE, Set A
128 rxns, Module 2
PN-1000539
Store at -20°C

	#	PN
BEAM Conjugate 9, PE	1	2000782
BEAM Conjugate 10, PE	1	2000783
BEAM Conjugate 11, PE	1	2000784
BEAM Conjugate 12, PE	1	2000785
BEAM Conjugate 13, PE	1	2000786
BEAM Conjugate 14, PE	1	2000787
BEAM Conjugate 15, PE	1	2000788
BEAM Conjugate 16, PE	1	2000789
Quenching Reagent	1	2000790

10x GENOMICS

Cap colors for BEAM Conjugates 1-16 tubes may vary depending on the lot. Verify reagent using the part number.

Chromium Human MHC Class I A0201 Monomer Kit, 32 rxns PN-1000542

Chromium
Human MHC Class I A0201 Monomer Kit
32 rxns, PN-1000542
Store at -20°C

	#	PN
Monomer A0201	1	2000802
Negative Control Peptide A0201	1	2000803
Dilution Buffer	1	2000801

10x GENOMICS

Chromium Human MHC Class I A1101 Monomer Kit, 32 rxns PN-1000543

Chromium
Human MHC Class I A1101 Monomer Kit
32 rxns, PN-1000543
Store at -20°C

	#	PN
Monomer A1101	1	2000804
Negative Control Peptide A1101	1	2000805
Dilution Buffer	1	2000801

10x GENOMICS

Chromium Human MHC Class I B0702 Monomer Kit, 32 rxns PN-1000544

Chromium
Human MHC Class I B0702 Monomer Kit
32 rxns, PN-1000544
Store at -20°C

	#	PN
Monomer B0702	1	2000806
Negative Control Peptide B0702	1	2000807
Dilution Buffer	1	2000801

10x GENOMICS

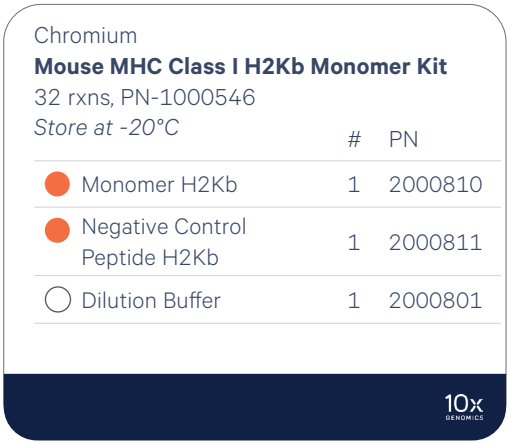
Chromium Human MHC Class I A2402 Monomer Kit, 32 rxns PN-1000545

Chromium
Human MHC Class I A2402 Monomer Kit
32 rxns, PN-1000543
Store at -20°C

	#	PN
Monomer A2402	1	2000808
Negative Control Peptide A2402	1	2000809
Dilution Buffer	1	2000801

10x GENOMICS

Chromium Mouse MHC Class I H2Kb Monomer Kit, 32 rxns PN-1000546



Protocol Steps & Timing

The table below provides an overview of the Barcode Enabled Antigen Mapping of antigen-specific TCRs (BEAM-T) protocol steps and timing.

Days	Steps	Timing	Stop & Store
Day 1	**Step 1. Pre-screen Peptides*** *(Includes preparing unquenched BEAM-T Assembly, sample labeling, and flow cytometric analysis)*	2-3 h	
Day 2	**Step 2. Test Multiple Peptides**		
	Step 2.1. BEAM-T Assembly Preparation	1 h	STOP 4°C ≤24 h
	Step 2.2. Sample Labeling	1 h	
	Step 2.3. Flow Sorting	2-3 h	

**Peptide pre-screening is required to determine the peptide quality and loading to monomers. This step can be skipped if the peptide is already pre-screened.*

*Keep samples on ice following sorting. Proceed **immediately** to the relevant Chromium Single Cell Immune Profiling Solutions User Guide with Feature Barcode technology (see References).*

Get Started

Cap colors for BEAM Conjugates 1-16 tubes may vary depending on the lot. Verify reagent using the part number.

Choose appropriate MHC monomer kit depending on the target peptide.

	Item	10x PN	Preparation & Handling	Storage
	Place on Ice			
●	**BEAM Conjugate 1-16, PE**	2000774 - 2000789	Thaw at room temperature. Vortex, centrifuge briefly, pipette mix 5x (pipette set at 20 µl), and place on ice.	–20°C
●	**Quenching Reagent**	2000790	Thaw at room temperature. Vortex, verify no precipitate, and centrifuge briefly. Place on ice	–20°C
○	**Dilution Buffer**	2000801	Thaw at room temperature. Vortex, verify no precipitate, and centrifuge briefly. Place on ice.	–20°C
	Peptide Use appropriate, high-quality MHC monomer-matched peptide.	—	Thaw at room temperature and place on ice. Dilute to 110 µM using sterile PBS.	–20°C
	Chromium Human MHC Class I A0201 Monomer Kit			
●	**Monomer A0201**	2000802	Thaw at room temperature. Centrifuge briefly, pipette mix 5x (pipette set at 100 µl), and place on ice.	–20°C
●	**Negative Control Peptide A0201**	2000803	Thaw at room temperature. Centrifuge briefly, pipette mix 5x (pipette set at 480 µl), and place on ice.	–20°C
	Chromium Human MHC Class I A1101 Monomer Kit			
●	**Monomer A1101**	2000804	Thaw at room temperature. Centrifuge briefly, pipette mix 5x (pipette set at 100 µl), and place on ice.	–20°C
●	**Negative Control Peptide A1101**	2000805	Thaw at room temperature. Centrifuge briefly, pipette mix 5x (pipette set at 480 µl), and place on ice.	–20°C
	Chromium Human MHC Class I B0702 Monomer Kit			
●	**Monomer B0702**	2000806	Thaw at room temperature. Centrifuge briefly, pipette mix 5x (pipette set at 100 µl), and place on ice.	–20°C
●	**Negative Control Peptide B0702**	2000807	Thaw at room temperature. Centrifuge briefly, pipette mix 5x (pipette set at 480 µl), and place on ice.	–20°C

	Chromium Human MHC Class I A2402 Monomer Kit			
	Monomer A2402	2000808	Thaw at room temperature. Centrifuge briefly, pipette mix 5x (pipette set at 100 µl), and place on ice.	–20°C
	Negative Control Peptide A2402	2000809	Thaw at room temperature. Centrifuge briefly, pipette mix 5x (pipette set at 480 µl), and place on ice.	–20°C
	Chromium Mouse MHC Class I H2Kb Monomer Kit			
	Monomer H2Kb	2000810	Thaw at room temperature. Centrifuge briefly, pipette mix 5x (pipette set at 100 µl), and place on ice.	–20°C
	Negative Control Peptide H2Kb	2000811	Thaw at room temperature. Centrifuge briefly, pipette mix 5x (pipette set at 480 µl), and place on ice.	–20°C
	T Cell Antibody Panel for Flow Sorting *See Appendix*	—	Manufacturer's recommendations.	4°C
	7-AAD (7-Aminoactinomycin D)	—	Manufacturer's recommendations.	4°C
	Fc Receptor Blocking Solution Human/Mouse	—	Manufacturer's recommendations.	—
	TotalSeq™-C Antibody Oligonucleotide Conjugates *Optional* *For simultaneous cell surface protein labeling*	—	Manufacturer's recommendations.	—
	Obtain			
	Sterile 1X PBS	—	—	4°C
	BSA *Optional* *For preparing PBS + 1% BSA for resuspending lyophillized antibody-oligonucleotide conjugates*	—	—	4°C
	Vortex	—	—	—
	FBS	—	Manufacturer's recommendations.	4°C

Peptide Dilution

Peptides working concentration should be 110 µM. If required, sterile PBS can be used for dilution. If using lyophilized peptide, resuspend in DMSO to a stock concentration of 10 mM at room temperature, then dilute using sterile PBS to a working concentration of 110 µM. Keep diluted peptide on ice during the assembly process. Unused peptide can be stored at -20°C.

Step 1: Pre-screen Peptides

Peptide Pre-screening

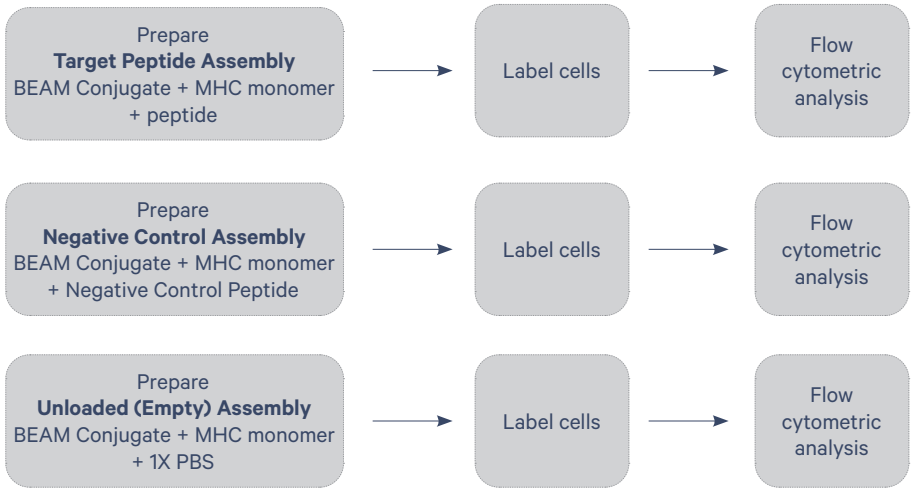
It is recommended to pre-screen each peptide before starting BEAM-T workflow to determine proper loading of the MHC monomer with the peptide. Improper MHC loading can alter PE signal during flow sorting.

This step only needs to be performed once. For peptides already screened, proceed to step 2.

Pre-screening does not require a positive signal and thus can be performed on non-experimental samples. For the purpose of pre-screening the peptides, the following three unique BEAM-T Assemblies should be prepared:

- Target Peptide Assembly
- Negative Control Assembly
- Unloaded (Empty) Assembly

Pre-screening Overview



BEAM-T Assembly for Pre-screening Peptides

TIPS

Assemblies can be prepared in a PCR 8-tube strip or a 1.5-ml microcentrifuge tube.

a. Target Peptide Assembly

i. Prepare Monomer-BEAM Conjugate Complex on ice in an appropriate tube. Add reagents in the order listed. Maintain on ice in the dark.

	BEAM Conjugate & Monomer Complex	PN	1 BEAM-T Assembly (µl)
○	**Dilution Buffer** *Pipette mix 5x slowly before adding. Buffer is prone to bubble formation.*	-	9.6
●	**BEAM Conjugate 1-16, PE**	2000774-2000789	2.4
	Monomer *Choose appropriate monomer*	-	3.0
	Total	**-**	**15.0**

Perform all pipette mixing steps without introducing bubbles.

ii. Pipette mix and dispense **10 µl** Monomer-BEAM Conjugate Complex into a tube. Keep the tubes on ice and in the dark.

iii. Add **5 µl** of 110 µM appropriate target peptide to 10 µl Monomer-BEAM Conjugate Complex. Pipette mix 5 - 10x.

b. Negative Control Assembly

i. Prepare Monomer-BEAM Conjugate Complex on ice in an appropriate tube. Add reagents in the order listed. Maintain on ice in the dark.

	BEAM Conjugate & Monomer Complex	PN	1 BEAM-T Assembly (µl)
○	**Dilution Buffer** *Pipette mix 5x slowly before adding. Buffer is prone to bubble formation.*	-	9.6
●	**BEAM Conjugate 1-16, PE**	2000774-2000789	2.4
	Monomer *Choose appropriate monomer*	-	3.0
	Total	**-**	**15.0**

ii. Pipette mix and dispense **10 µl** Monomer-BEAM Conjugate Complex into a tube. Keep the tube on ice and in the dark.

iii. Add **5 µl** appropriate Negative Control Peptide to 10 µl Monomer-BEAM Conjugate Complex. Pipette mix 5 - 10x.

c. Unloaded (Empty) Assembly

i. Prepare Monomer-BEAM Conjugate Complex on ice in an appropriate tube. Add reagents in the order listed. Maintain on ice in the dark.

BEAM Conjugate & Monomer Complex	PN	1 BEAM-T Assembly (µl)
○ **Dilution Buffer** *Pipette mix 5x slowly before adding. Buffer is prone to bubble formation.*	-	9.6
● **BEAM Conjugate 1-16, PE**	2000774-2000789	2.4
Monomer *Choose appropriate monomer*	-	3.0
Total	**-**	**15.0**

ii. Pipette mix and dispense **10 µl** Monomer-BEAM Conjugate Complex into a tube. Keep the tube on ice and in the dark.

iii. Add **5 µl** PBS to 10 µl Monomer-BEAM Conjugate Complex. Pipette mix 5 - 10x.

STOP

d. Incubate the assemblies for **30 min** on ice in the dark or store the BEAM-T Assemblies up to **24 h** at **4°C** in the dark.

e. To minimize the bubble formation, centrifuge the assemblies at **2,500 rcf** for **5 min** at **4°C**.

f. Place on ice in the dark and proceed **immediately** to the next step.

Sample Labeling & Flow Cytometry

This protocol was demonstrated using 0.2-1 x 10^6 cells. Wash and thaw cells according to the appropriate 10x Genomics Demonstrated Protocol available on the 10x Genomics Support website.

a. Prepare PBS + 2% FBS and keep on ice.

b. Prepare T cell antibody panel at appropriate dilution and keep on ice in the dark. See Appendix for examples panels for human and mouse.

c. Dispense 0.2-1 x 10^6 cells each into a new 15-ml centrifuge tube for each Target Peptide Assembly, Negative Control Assembly, and Unloaded (Empty) Assembly.

d. Centrifuge cells at **300 rcf** for **5 min** at **4°C**.

e. Remove the supernatant.

f. Resuspend each pellet in **90 µl** chilled PBS + 2% FBS and keep on ice.

g. Add **10 µl** human/mouse Fc Receptor Blocking Solution to each tube, mix and incubate for **10 min** on ice.

h. Add the entire volume (~**15 µl**) of appropriate BEAM-T Assembly to the cells and label the tubes accordingly. Using a P1000 pipette, gently mix 5x.

i. Incubate for **15 min** on ice in the dark.

j. Add appropriate volume of T cell antibody panel for flow cytometry to the cells and pipette mix. See Appendix for example panels.

k. Incubate for **30 min** on ice in the dark and gently pipette mix cells every 10 min.

l. Add **3.5 ml** chilled PBS + 2 % FBS. Gently pipette mix 5x.

m. Centrifuge at **300 rcf** for **5 min** at **4°C**.

n. Remove the supernatant.

o. Repeat l - n 2x for a total of three washes.

p. Resuspend cells in **100 µl** chilled PBS + 2% FBS. Pipette mix 5x.

q. Add **0.5 µl** 7-AAD per **100 µl** sample.

r. Maintain sample on ice in dark and proceed to flow cytometry.

Flow Cytometry

a. Prepare appropriate compensation controls. Compensation controls should be prepared fresh for every flow sorting experiment. For further details, consult Technical Note Barcode Enabled Antigen Mapping (BEAM) Flow Cytometry Guidelines (Document CG000598).

b. Prepare fluorescence minus one (FMO) controls. See Appendix for details.

c. Gate on lymphocytes by size (scatter), single cells, live cells (e.g. 7-AAD negative), lineage negative, $CD8^+$ cells, and then analyze dual positive PE^+ $CD8^+$ cells.

d. Analyze the results and proceed with step 2 if the peptide passes the pre-screening. See Appendix for Peptide Pre-screening Analysis for BEAM-T.

Step 2: Test Multiple Peptides

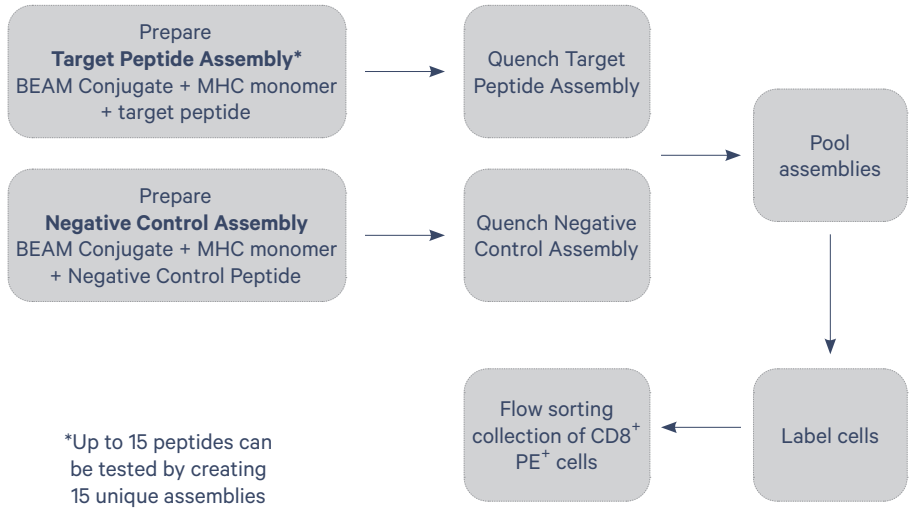
Step 2.1: BEAM-T Assembly Preparation

This section provides guidance on testing multiple peptides in a sample. A downloadable workbook (Barcode Enabled Antigen Mapping (BEAM) Workbook for BEAM-T Assembly, Document CG000615) for the calculations described in this section is also available on the 10x Genomics Support website and can be used concurrently with this User Guide.

For the purpose of testing multiple peptides, the following two types of BEAM-T Assemblies should be prepared:

i. One or more Target Peptide Assembly
ii. Negative Control Assembly

Testing Multiple Peptides Overview



BEAM-T Assembly Volume

Each final quenched BEAM-T Assembly is 30 µl and is sufficient to label up to 1 x 10^6 cells (200,000 to 1 x 10^6). To label more than 1 x 10^6, the final volume of the BEAM assemblies will need to be scaled.

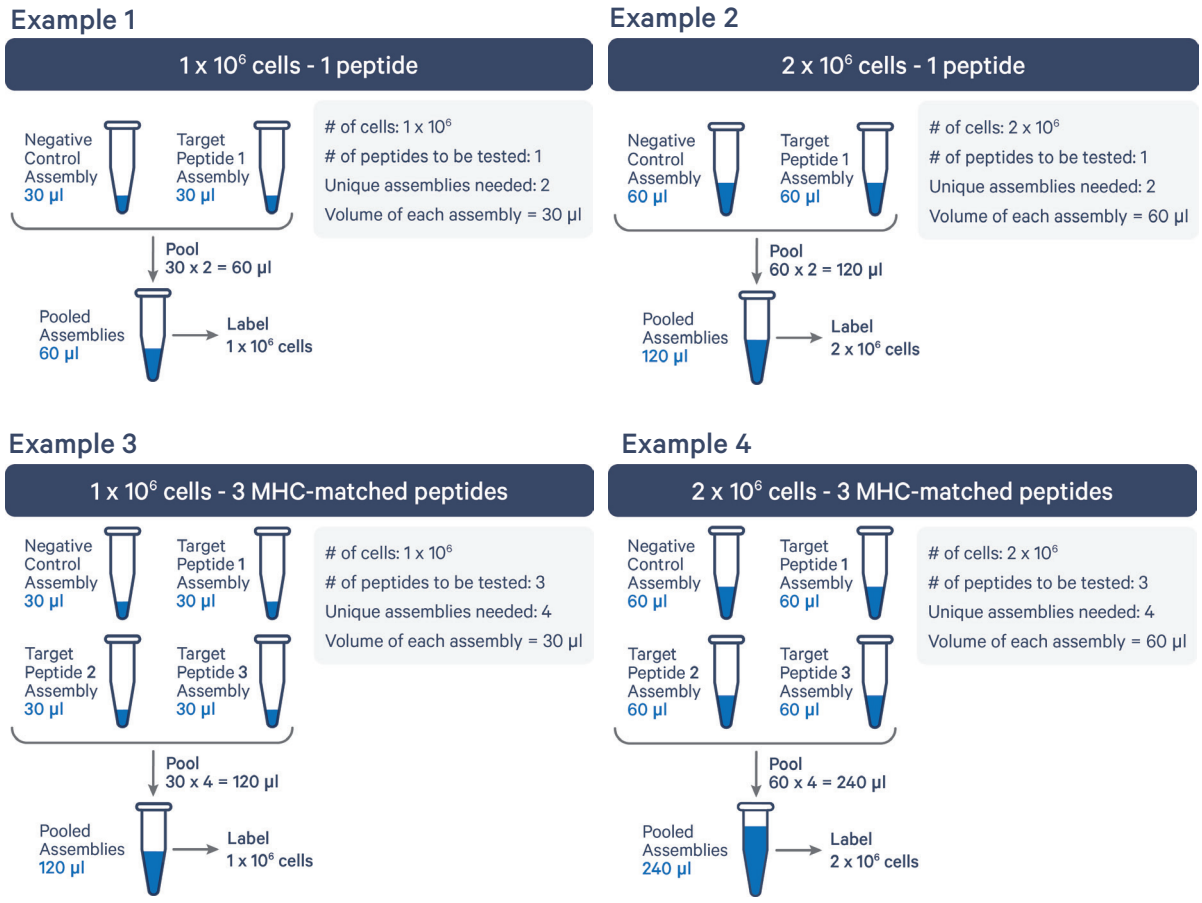
Number of Cells	Volume of each BEAM-T Assembly (µl)
200,000 - 1 x 10^6	30
1 x 10^6 - 2 x 10^6	60
2 x 10^6 - 3 x 10^6	90
3 x 10^6 - 4 x 10^6	120
4 x 10^6 - 5 x 10^6	150
5 x 10^6 - 6 x 10^6	180
6 x 10^6 - 7 x 10^6	210
7 x 10^6 - 8 x 10^6	240

Each of the BEAM Conjugate provided is sufficient to label up to 8 x 10^6 cells. If working with >8 x 10^6 cells, the same peptide can be assembled on a different BEAM Conjugate.

BEAM-T Assembly Setup Examples

The figures below provide some possible BEAM-T assembly setup examples when starting with 1 x 10^6 and 2 x 10^6 cells. Note that the final volume of each unique assembly is scaled up when labeling more than 1 x 10^6 cells (examples 2 and 4).

For testing 15 target peptides in a sample containing 1 x 10^6 cells, 16 unique BEAM-T Assemblies (includes one negative control reaction), each containing a unique BEAM Conjugate and a unique peptide, would be needed. In this case, each assembly would have a final volume of 30 µl. If the number of cells is more than 1 x 10^6 cells, the volume of each BEAM-T Assembly should be scaled up accordingly. See BEAM-T Assembly Volume table on the previous page.



In these examples, same monomer is being used. If using more than one monomer in an experiment, set up a separate Negative Control Assembly for each monomer.

TIPS

Assemblies can be prepared in a PCR 8-tube strip or a 1.5-ml microcentrifuge tube.

a. Target Peptide Assembly

i. Prepare Monomer-BEAM Conjugate Complex on ice in an appropriate tube and pipette mix. Add reagents in the order listed. Maintain on ice in the dark.

BEAM Conjugate & Monomer Complex	PN	1 BEAM-T Assembly (µl)
○ **Dilution Buffer** *Pipette mix 5x slowly before adding. Buffer is prone to bubble formation.*	-	9.6
● **BEAM Conjugate 1-16, PE**	2000774-2000789	2.4
Monomer *Choose appropriate monomer*	-	3.0
Total	**-**	**15.0**

Barcode Enabled Antigen Mapping (BEAM) Workbook for BEAM-T Assembly (Document CG000615) can also be used for calculations relevant to creating multiple BEAM-T Assemblies.

ii. Pipette mix and dispense **10 µl** Monomer-BEAM Conjugate Complex into a tube. Keep the tubes on ice and in the dark.

iii. Add **5 µl** of 110 µM appropriate target peptide to 10 µl Monomer-BEAM Conjugate Complex. Pipette mix 5 - 10x.

b. Negative Control Assembly

i. Prepare Monomer-BEAM Conjugate Complex on ice in an appropriate tube and pipette mix. Add reagents in the order listed. Maintain on ice in the dark.

BEAM Conjugate & Monomer Complex	PN	1 BEAM-T Assembly (µl)
○ **Dilution Buffer** *Pipette mix 5x slowly before adding. Buffer is prone to bubble formation.*	-	9.6
● **BEAM Conjugate 1-16, PE**	2000774-2000789	2.4
Monomer *Choose appropriate monomer*	-	3.0
Total	**-**	**15.0**

ii. Pipette mix and dispense **10 µl** Monomer-BEAM Conjugate Complex into a tube. Keep the tube on ice and in the dark.

iii. Add **5 µl** appropriate Negative Control Peptide. Pipette mix 5 - 10x.

STOP

c. Incubate for **30 min** on ice in the dark or store the BEAM-T Assemblies up to **24 h** at **4°C** in the dark.

d. Prepare Quenching Mix on ice in an appropriate tube and pipette mix. Quenching mix should be prepared 5-10 min before using.

Quenching Mix *Add reagents in the order listed. Maintain on ice.*	PN	1 BEAM-T Assembly (µl)	4 BEAM-T Assemblies + 10% (µl)	8 BEAM-T Assemblies + 10% (µl)
Quenching Reagent	2000790	6.0	26.4	52.8
Negative Control Peptide *Use appropriate MHC-matched Negative Control Peptide*	-	9.0	39.6	79.2
Total	-	**15.0**	**66.0**	**132.0**

Barcode Enabled Antigen Mapping (BEAM) Workbook for BEAM-T Assembly (Document CG000615) can also be used for calculations relevant to multiple BEAM-T Assemblies.

e. Add **15 µl** Quenching Mix to each tube containing BEAM-T Assembly (negative control as well as target peptides).

f. Pipette mix 10x and incubate for **15 min** on **ice** in the dark.

g. To minimize the bubble formation, centrifuge the quenched assemblies at **2,500 rcf** for **5 min** at **4°C**.

h. Pool the entire volume (~30 µl per assembly per 1 x 10^6 cells) of each unique Target Peptide Assembly (if working with more than one peptide) and entire volume (~30 µl per 1 x 10^6 cells) of the Negative Control Peptide Assembly in a tube. Pipette mix and maintain at 4°C in dark.

30 µl of the final quenched assembly is needed to label 1 x 10^6 cells. If working with more than 1 x 10^6 cells, scaled up volume of each BEAM-T Assembly will be needed for pooling. See BEAM-T Assembly Volume table. The volume of assembly should be scaled up for every 1 x 10^6 total cells, regardless of the expected percentage of T cells in the sample.

i. **Immediately** proceed to step 2.2 Sample Labeling.

Step 2.2: Sample Labeling

This protocol was demonstrated using 0.2-8 x 10^6 cells. For more than 8 x 10^6 cells, set up additional labeling reactions. Wash and thaw cells according to the appropriate 10x Genomics Demonstrated Protocol available on the 10x Genomics Support website.

a. Prepare PBS + 2% FBS and keep on ice. This buffer can be prepared a day before the experiment.

b. Prepare T cell antibody panel at appropriate dilution and keep on ice in the dark. See Appendix for some examples panels.

c. **Optional Step** Prepare optional Antibody Mix Supernatant for optional cell surface protein labeling and keep on ice.

Prepare	
Antibody Mix Supernatant (containing antibody-oligonucleotide conjugates) *For optional cell surface protein labeling*	• Add appropriate/manufacturer's recommended amount of antibody-oligonucleotide conjugates to a 1.5-ml microcentrifuge tube. • If using a custom lyophillized antibody: Resuspend the antibody-oligonucleotide conjugates in an appropriate volume of PBS + 1% BSA. • Centrifuge the mix at 14,000 rcf for 10 min at 4°C. • Transfer the supernatant (containing Antibody Mix) to a new tube and maintain at 4°C.

d. Dispense 0.2-8 x 10^6 cells into a new 15-ml centrifuge tube.

e. Centrifuge at **300 rcf** for **5 min** at **4°C**.

f. Remove the supernatant.

g. Resuspend pellet in **90 µl** chilled PBS + 2% FBS and keep on ice.

h. Add appropriate volume of human/mouse Fc Receptor Blocking Solution (10 µl for up to 1 x 10^6 cells), pipette mix, and incubate for **10 min** on ice. If working with more than 1 x 10^6 cells, scale up the volume of blocking solution accordingly.

i. Add the entire volume of pooled assemblies from step 2.1 to the cells. Using a P1000 pipette, gently mix 5x.

j. Incubate for **15 min** at **4°C** in the dark.

k. Add appropriate volume of T cell antibody panel for flow sorting (and Antibody Mix Supernatant, if applicable) to the cells directly and pipette mix.

l. Incubate for **30 min** at **4°C** in the dark and gently pipette mix cells every 10 min.

m. Add **3.5 ml** chilled PBS + 2 % FBS. Gently pipette mix 5x.

n. Centrifuge at **300 rcf** for **5 min** at **4°C**.

o. Remove the supernatant.

p. **Repeat** m - o 2x for a total of three washes.

q. Resuspend cells in appropriate volume chilled PBS + 2% FBS. Pipette mix 5x.
Resuspension volume depends on the number of the cells. Resuspend in 100 µl for every 1 x 10^6 cells labeled. For example, for 8 x 10^6 labeled cells, resuspend pellet in 800 µl chilled PBS + 2% FBS.

r. Add **1.0 µl** 7-AAD per **200 µl** sample.

s. Maintain sample on ice in the dark and proceed **immediately** to flow sorting with appropriate settings.

Step 2.3: Flow Sorting

a. Prepare PBS + 20% FBS for pre-coating the collection tubes.

b. Prepare Collection Buffer for sorted cells and maintain the buffer on ice.
See the table below for guidance on choosing the appropriate Collection Buffer. This table also provides guidance on the appropriate volume for collection and post-sorting steps.

Collection Buffers and Post-Sorting Guidance

Collection Buffer	Volume for # cells sorted	Expected cell conc. (confirm by counting)	Centrifugation to concentrate cells post-sorting?	Post-sorting notes
PBS + 5% FBS	20 µl for ≤5,000 cells	≤147 cells/µl	No	This volume can be accommodated in 1 chip well
PBS + 5% FBS	20 µl for ≤10,000 cells	≤208 cells/µl	No	This volume must be loaded in 2 chip wells
PBS + 20% FBS	20 µl for ≤20,000 cells	≤263 cells/µl	No	This volume must be loaded in 2 chip wells
PBS + 20% FBS	30 µl for 20,001-50,000 cells	232-294 cells/µl	No	This volume must be loaded in 4 chip wells
PBS + 20% FBS	30 µl for 50,001-500,000 cells	–	Recommended	• After sorting, add additional cold PBS+20% FBS for a total 1.5 ml volume. • Centrifuge at 150 rcf for 10 min at 4°C. • Count
100% FBS	30 µl for more than 500,000 sorted cells	–	Yes	• After sorting, add additional cold PBS+20% FBS for a total 1.5 ml volume. • Centrifuge at 150 rcf for 10 min at 4°C. • Count

When working with small numbers of cells (e.g., less than 50,000), expect poor (<75%) recovery of cells by centrifugation.

c. Pre-coat sorting collection tubes by adding **1 ml** PBS + 20% FBS to a tube then removing all of the PBS + 20% FBS.

d. Add an appropriate Collection Buffer (see the table above) to the collection tube.

e. Prepare appropriate compensation controls. Compensation controls should be prepared fresh for every flow sorting experiment. For further details, consult the Technical Note Barcode Enabled Antigen Mapping (BEAM) Flow Cytometry Guidelines (Document CG000598).

f. Prepare fluorescence minus one (FMO) controls. See Appendix for details.

g. Gate on lymphocytes by size (scatter), single cells, live cells (e.g. 7-AAD negative), lineage negative, $CD8^+$ cells, and then sorting dual positive PE^+ $CD8^+$ cells.

h. Cells should be sorted using the purity mode on a low pressure setting (e.g. 100 µM nozzle, etc). Keep cells on ice and in the dark prior to sorting. During sorting, the collection tube should be chilled. Place the sorted cells immediately on ice after sorting.

Consult the Technical Note Barcode Enabled Antigen Mapping (BEAM) Flow Cytometry Guidelines (Document CG000598) for details on gating strategy and other cell sorting best practices.

i. Determine the cell concentration and viability using an automated cell counter or a hemocytometer. Cell counting can be skipped if the sorted cell numbers are expected to be low.

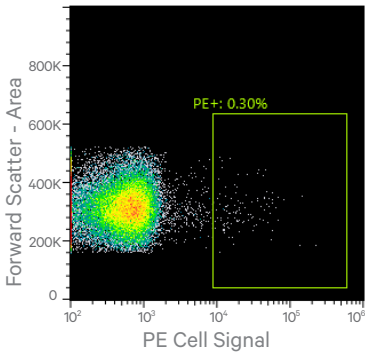
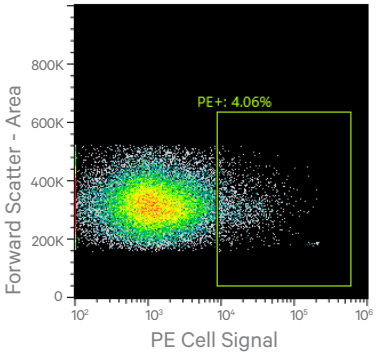
If necessary, the collected cells may be concentrated by centrifugation at 150 rcf for 10 min at 4°C and by removing the supernatant (see Collection Buffers and Post-Sorting Guidance table). Use of a longer centrifugation time is recommended post sorting.

j. Proceed **immediately** to the relevant Chromium Single Cell Immune Profiling Solutions User Guide with Feature Barcode technology (see References).

10x GENOMICS

Troubleshooting

Troubleshooting Guide

Issue	Potential Causes	Mitigation Strategies
Background PE signal is observed with cells labeled with the Negative Control BEAM-Ab Assembly **Normal**  **High Background**  	• Using BSA-containing buffers • Using bead based kits for pre-enrichment • Insufficient washing prior to flow analysis • Not performing quenching during BEAM-Ab pre-screening	• Prepare the BEAM-Ab Negative Control Assembly using an off-target antigen instead of PBS. • Use chilled PBS + 2% FBS for labeling and washing • DO NOT use alternative buffers. BSA-containing buffers may increase background. • Perform three wash steps with 3.5 ml buffer and pipette mix 5x. DO NOT reduce the number or volume of wash steps. • Ensure that quenching is performed during BEAM-Ab pre-screening.

Appendix

A1. Antigen Pre-screening Analysis for BEAM-Ab

The flow plots are representative. The gates shown here are specific to the sorter settings and the sample used in that experiment and can vary between experiments. Absolute values may vary depending upon sorter setting and the sample used.

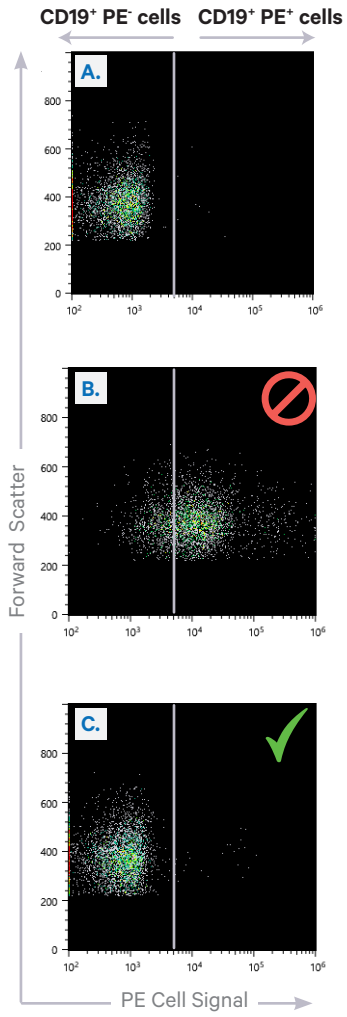
a. Run the Negative Control Assembly through the flow cytometer to establish a region that can be used to identify and gate for positive cells.

Figure A. Sample labeled with Negative Control Assembly.

b. Next, run each Target Antigen Assembly and use the same gating scheme as the Negative Control Assembly. See examples below:

Figure B. A low quality antigen in a sample containing no or few target positive cells might show a shift of the whole population to the right.

Figure C. A good quality antigen in a sample containing no or few target positive cells will look very similar to the Negative Control Assembly flow plot as above.



The plots show PE intensity of $CD19^+$ cells

A2. Peptide Pre-screening Analysis for BEAM-T

The flow plots are representative. The gates shown here are specific to the sorter settings and the sample used in that experiment and can vary between experiments. Absolute values may vary depending up on the sorter setting and the sample used.

a. Run the Negative Control Assembly through the flow cytometer to establish a region that can be used to identify and gate for positive cells.

Figure A. Sample labeled with a Negative Control Assembly.

b. Next, run the Unloaded (Empty) Assembly and use the same gating scheme as the Negative Control Peptide Assembly.

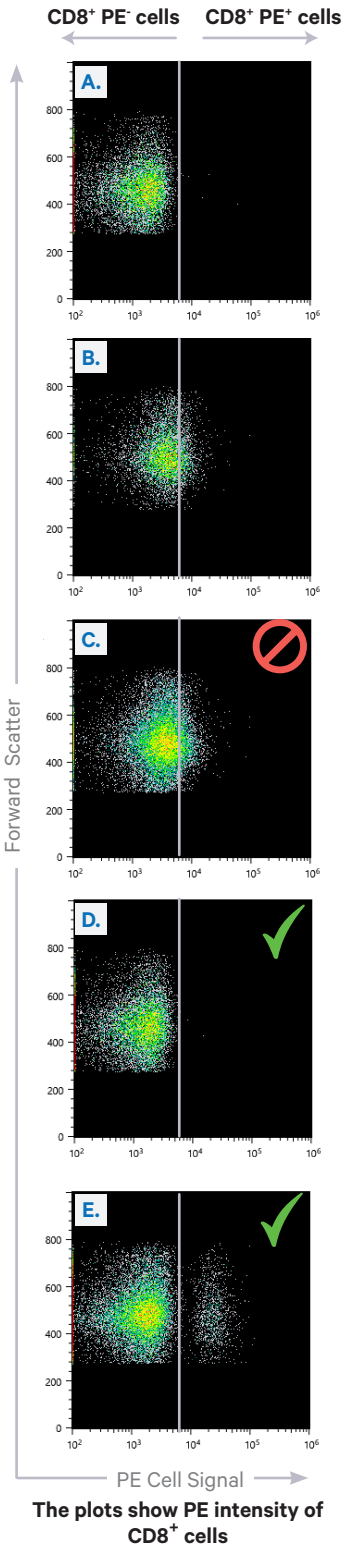
Figure B. Sample labeled with an Unloaded (Empty) Assembly.

c. Finally, run each Target Peptide Assembly. Use the same gating scheme as the Negative Control Assembly and compare with the unloaded assembly. See examples below:

Figure C. Example of a poorly loaded peptide in a sample containing no target positive cells. The sample might show a shift of the whole population to the right and will appear similar to the Unloaded (Empty) Assembly.

Figure D. Example of a properly loaded peptide in a sample containing no target positive cells. Sample will appear very similar to the negative control flow plot as above.

Figure E. Example of a properly loaded peptide in a sample containing known target positive cells. In this case, the center of the negative population is similar to the negative control above.



The plots show PE intensity of $CD8^+$ cells

A3. BEAM-Ab Antibody Panels for Flow Sorting

The following tables provide example panels for flow sorting human and mouse B cells. The purpose is to sort lymphocytes > single cells > live cells (7-AAD negative) > lineage negative > $CD19^+$ > dual $CD19^+PE^+$ cells.

Example Human B Cell Antibody Panel for Flow Sorting

Marker	Target Cell Type	Antibody	Vendor	Part Number (US)
CD19 (+)	B cells	PE/Cyanine7 anti-human CD19 Antibody	Biolegend	302215
CD56 (-)	Natural killer cells	Brilliant Violet 421 anti-human CD56 (NCAM) Antibody	Biolegend	318327
CD3 (-)	T cells	Brilliant Violet 421 anti-human CD3 Antibody	Biolegend	317344
CD14 (-)	Monocytes	Brilliant Violet 421 anti-human CD14 Antibody	Biolegend	367143
*7-AAD (-)	Live dead marker	Invitrogen eBioscience 7-AAD Viability Staining Solution	Invitrogen	00699350

Example Mouse B Cell Antibody Panel for Flow Sorting

Marker	Target Cell Type	Antibody	Vendor	Part Number (US)
CD19 (+)	B cells	PE/Cyanine7 anti-mouse CD19 Antibody	Biolegend	115519
Ter119 (-)	Erythroid cells	Brilliant Violet 421 anti-mouse TER-119/Erythroid Cells Antibody	Biolegend	116233
Ly6g (-)	Myeloid cells	Brilliant Violet 421 anti-mouse Ly-6G/Ly-6C (Gr-1) Antibody	Biolegend	108433
CD3 (-)	T cells	Brilliant Violet 421 anti-mouse CD3 Antibody	Biolegend	100227
CD14 (-)	Dendritic cells	Brilliant Violet 421 anti-mouse CD14 Antibody	Biolegend	123329
*7-AAD (-)	Live dead marker	Invitrogen eBioscience 7-AAD Viability Staining Solution	Invitrogen	00699350

**7-AAD is added separately just before flow sorting.*
Refer to the manufacturer's website for regional part numbers. Same antibodies can also be used when performing pre-screening.

A4. BEAM-T Antibody Panels for Flow Sorting

The following tables provide example panels for flow sorting human and mouse T cells. The purpose is to sort lymphocytes > single cells > live cells (7-AAD negative) > lineage negative > $CD8^+$ > dual $CD8^+PE^+$ cells.

Example Human T Cell Antibody Panel for Flow Sorting

Marker	Target Cell Type	Antibody	Vendor	Part Number (US)
CD3 (+)	Total T cells	Brilliant Violet 510 anti-human CD3 Antibody	Biolegend	317331
CD8 (+)	Cytotoxic T cells	Alexa Fluor 488 anti-human CD8 Antibody	Biolegend	344716
CD56 (-)	Natural killer cells	Brilliant Violet 421 anti-human CD56 (NCAM) Antibody	Biolegend	318327
*7-AAD (-)	Live dead marker	Invitrogen eBioscience 7-AAD Viability Staining Solution	Invitrogen	00699350

Example Mouse T Cell Flow Antibody Panel for Flow Sorting

Marker	Target Cell Type	Antibody	Vendor	Part Number (US)
CD3 (+)	Total T cells	Brilliant Violet 510 anti-mouse CD3 Antibody	Biolegend	100233
CD8 (+)	Cytotoxic T cells	FITC anti-Mouse CD8a Antibody	BD Bioscience	553030
Ter119 (-)	Erythroid cells	Brilliant Violet 421 anti-mouse TER-119/Erythroid Cells Antibody	Biolegend	116233
Ly6g (-)	Myeloid cells	Brilliant Violet 421 anti-mouse Ly-6G/Ly-6C (Gr-1) Antibody	Biolegend	108433
CD19 (-)	B cells	Brilliant Violet 421 anti-mouse CD19 Antibody	Biolegend	115537
CD14 (-)	Dendritic cells	Brilliant Violet 421 anti-mouse CD14 Antibody	Biolegend	123329
*7-AAD (-)	Live dead marker	Invitrogen eBioscience 7-AAD Viability Staining Solution	Invitrogen	00699350

**7-AAD is added separately just before flow sorting.*
Refer to the manufacturer's website for regional part numbers. Same antibodies can also be used when performing pre-screening.

A5. FMO Controls

BEAM-Ab

BEAM-T

This section provides guidance on preparing FMO controls for Barcode Enabled Antigen Mapping of antigen-specific BCRs (BEAM-Ab) and antigen-specific TCRs (BEAM-T) protocols.

FMO control should be prepared fresh for every flow sorting experiment. Cells should be labeled with the antibody panel and live dead marker but not the BEAM assembly. If enough cells from the experimental sample are not available, cells from a non-experimental sample can be used.

a. Dispense 100,000 cells into a new 1.5-ml microcentrifuge tube

b. Centrifuge at **300 rcf** for **5 min** at **4°C**.

c. Remove the supernatant.

d. Resuspend pellet in **90 µl** chilled PBS + 2% FBS and keep on ice.

e. Add **10 µl** human/mouse Fc Receptor Blocking Solution and incubate for **10 min** on ice

f. Add appropriate volume of B/T cell antibody panel to the cells.

g. Incubate for **30 min** on ice in the dark.

h. Add **3.5 ml** chilled PBS + 2% FBS. Gently pipette mix 5x.

i. Centrifuge at **300 rcf** for **5 min** at **4°C**.

j. Remove the supernatant.

k. **Repeat** h-j one more time for a total of two washes.

l. Resuspend cells in **100 µl** chilled PBS + 2% FBS. Pipette mix 5x.

m. Add **0.5 µl** 7-AAD (live dead marker) per **100 µl** sample.

n. Maintain sample on ice in dark and proceed immediately to flow sorting

A6. References

Chromium Single Cell Immune Profiling Solutions User Guides with Feature Barcode technology:

If the cells were also labeled with antibody-oligonucleotide conjugates:

- Chromium Next GEM Single Cell 5' v2 (Dual Index) Reagent Kits with Feature Barcode technology for Barcode Enabled Antigen Mapping (BEAM) and Cell Surface Protein User Guide (CG000592).
- Chromium Next GEM Single Cell 5' HT v2 (Dual Index) Reagent Kits with Feature Barcode technology for Barcode Enabled Antigen Mapping (BEAM) and Cell Surface Protein User Guide (CG000594).

If the cells were not labeled with antibody-oligonucleotide conjugates:

- Chromium Next GEM Single Cell 5' v2 (Dual Index) Reagent Kits with Feature Barcode technology for Barcode Enabled Antigen Mapping (BEAM) User Guide (CG000591).
- Chromium Next GEM Single Cell 5' HT v2 (Dual Index) Reagent Kits with Feature Barcode technology for Barcode Enabled Antigen Mapping (BEAM) User Guide (CG000593).