

# Corning® X-MINI® Human CD8 Selection Kit

## Instructions for Use

CORNING

### General Information

#### Kit Contents (Cat. No. 6910)

Component Name	Quantity	Format	Storage
Biotin Mouse Anti-Human CD8 Antibody	2 mL vial	Isotype: Mouse IgG 1, kappa; buffer solution containing BSA and 0.09% sodium azide	Store 2°C to 8°C <b>DO NOT FREEZE</b>
Corning X-BACS™ Reagent	1 vial	Lyophilized	Store 2°C to 8°C <b>DO NOT FREEZE</b>
Corning X-BACS Buffer	1 x 125 mL	DPBS, 2 mM EDTA, [-] Ca <sup>2+</sup> [-] Mg <sup>2+</sup>	Store 2°C to 30°C
Syringe, 10 mL	1	Sterile	Room Temperature
21G x 1 ½" Needle	2	Sterile	Room Temperature
Syringe cap	10	Sterile	Room Temperature

Product capacity: ~3.6 x 10<sup>8</sup> PBMCs

All components are single-use only. Expiration dates are indicated on the component labeling.

#### Symbol Legend



Caution



Refer to instructions



Lot Number



Storage Temperature



Manufactured By



Keep Dry



Catalog Number



Expiration Date

#### Description

The X-MINI CD8 Selection Kit is intended for the selection of human CD8<sup>+</sup> cells from a peripheral blood mononuclear cell (PBMC) fraction. The following protocol details the selection of CD8<sup>+</sup> cells using a biotinylated antibody conjugate. Selected cells are immediately available for downstream applications such as flow cytometry and cell culture expansion.

#### Principle

The basic target cell selection is completed in four steps:

1. Targeting of CD8<sup>+</sup> cells from a prepared PBMC fraction using a biotinylated CD8 antibody.
2. Binding of streptavidin-coated microbubbles to biotinylated-target cells using proprietary X-BACS technology.
3. Separation of target and non-target cells using centrifugation. Collection of microbubble fraction using standard lab techniques.
4. Removal of microbubbles from target cells using positive pressure to retrieve the target cell fraction.

#### Required Buffer





Supplement X-BACS Buffer to achieve a final buffer concentration of 1% BSA or HSA, 2 mM EDTA in Ca<sup>2+</sup> and Mg<sup>2+</sup> free DPBS.

**NOTE:** Refrigerate X-BACS Buffer at 2°C to 8°C after supplementation.

### Materials Needed (not supplied)

- ▶ Corning® X-MINI® Pressor (Corning 6915)
- ▶ 10 mL syringe (BD Biosciences 309604)
- ▶ Syringe caps (BD Biosciences 305819)
- ▶ BSA (Sigma A3299)
- ▶ Cell strainer (optional; e.g., Corning 352340 or 431750)
- ▶ 2 mL microcentrifuge tubes (e.g., Corning MCT-200-C-S)
- ▶ Rotating mixer (e.g., Labnet H5600 or H5500)
- ▶ Saline (0.9% NaCl)

### Precautions

-  Follow standard laboratory procedures when working with biological material, sharps, and biohazardous waste.
-  Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
-  Reagents contained in this kit contain bovine serum albumin obtained from a commercial supplier. Information regarding the origin of the animal source used are maintained by the supplier and made available to Corning. The position statements provided by the supplier state the sources of bovine material origin are from the United States, Canada, and Australia.
-  The safety and effectiveness of this device for *in vivo* indications for use has not been established.

### Preparation of PBMC Sample and Corning X-BACS™ Reagent

#### Preparation of PBMC

1. Prepare a single cell suspension of PBMC from whole blood by traditional methods or by using the Corning X-LAB® System. **For Optimal Results:** Filter aggregated suspensions through a cell strainer.
2. After preparation, resuspend cells in X-BACS Buffer at a cell density of up to  $3 \times 10^7$  cells/mL.

#### Preparation of Corning X-BACS Reagent

**Note:** Before use, allow 15 minutes for X-BACS Reagent to come to room temperature (20°C to 25°C).

1. Insert the provided 21G x 1 ½" needle through the septum of the X-BACS Reagent as a vent for pressure compensation.
2. Using the provided syringe and the second 21G x 1 ½" needle, rapidly add 6 mL of saline to the vial.
3. Remove the syringe and vent needle from the vial.
4. Vigorously shake X-BACS Reagent for 10 seconds, and let the vial sit at room temperature for 5 minutes.

5. Prior to use, vigorously mix the X-BACS Reagent for 10 seconds then insert the 21G x 1 ½" needle through the septum for pressure compensation. Turn the vial upside down and insert a second 21G x 1 ½" needle attached to a syringe to recover the desired volume of microbubbles.

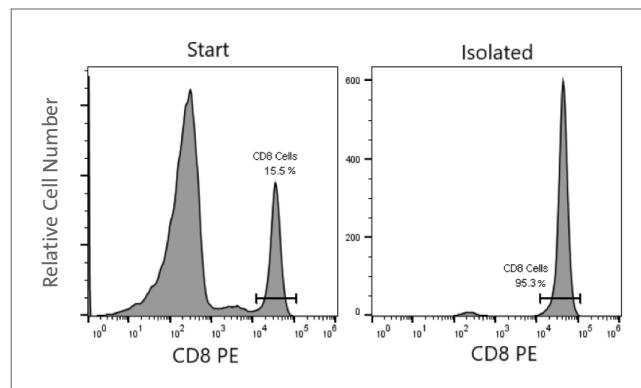


## CD8 Cell Selection

Cell labeling and selection volumes are for up to  $3 \times 10^7$  total cells per PBMC sample. When working with cell numbers above  $3 \times 10^7$  cells scale up all reagent volumes and total volumes accordingly.

	Instructions	PBMC
1.	Mix sample preparation thoroughly and transfer cells to a 2 mL microcentrifuge tube	1 mL
2.	Add indicated volume CD8 antibody for each 1 mL sample.	100 $\mu$ L
3.	Mix at room temperature and incubate using a rotating mixer.	30 minutes
4.	After the incubation period, mix the Corning® X-BACS™ Reagent and add indicated volume to each sample tube.	500 $\mu$ L
5.	Mix at room temperature and incubate using a rotating mixer to bind the microbubbles to the antibody-labeled cells.	20 minutes
6.	Centrifuge cells for separation.	5 minutes at 400 x g
7.	During centrifugation, prepare syringe for degassing of the microbubbles. Remove the plunger from the 10 mL syringe and attach a syringe cap. <b>NOTE:</b> Do not reinsert the plunger at this step.	
8.	Carefully pipette off white microbubble layer with a 200 $\mu$ L pipet and transfer to a pre-capped syringe. <b>NOTE:</b> Transfer of the microbubble layer will require multiple transfers with the pipet.	
9.	Use X-BACS Buffer to wash any residual microbubbles adhering to the sides of the sample tube or pipet tip and add to the pre-capped syringe. <b>NOTE:</b> Do not disturb the cell pellet. The remaining pelleted cells are the unwanted (negative) cell fraction.	
10.	Increase the volume of the pre-capped 10 mL syringe containing the microbubble layer to a final volume of 3 mL with X-BACS Buffer. Replace the plunger.	
11.	Mix the sample thoroughly by inversion. Immediately proceed to the next step.	
12.	Degassing of the microbubble can be done using the Corning X-MINI® Pressor or manually.	
	<b>Corning X-MINI Pressor:</b> Follow the X-MINI Pressor product insert instructions. In brief: lift the handle and securely attach pre-capped syringe into the syringe holder. Place the cover firmly over the syringe and pull lever down to degas the microbubble-cell sample for 30 seconds. Lift the lever handle when done .	
	<b>Manual Degassing:</b> Quickly and forcefully press the plunger down and hold for 30 seconds.	
13.	Repeat Steps 11 and 12 (for a total of 2 x 30-second degassing steps). The suspension will become less opaque.	
14.	The selected cells are now ready for analysis and downstream applications. <b>NOTE:</b> For cell culture, wash cells and resuspend in complete media prior to culturing.	

## Corning X-MINI Human CD8 Selection Kit Data Example



Starting from human PBMCs, CD8<sup>+</sup> cells were selected using biotinylated CD8 antibody and Corning X-BACS technology. The cells were fluorescently stained with CD8-PE. Cell debris and dead cells were excluded from analysis.

For more specific information on claims, visit the Certificates page at [www.corning.com/lifesciences](http://www.corning.com/lifesciences).

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# CORNING

**Corning Incorporated**  
*Life Sciences*

836 North St.  
Building 300, Suite 3401  
Tewksbury, MA 01876  
t 800.492.1110  
t 978.442.2200  
f 978.442.2476

[www.corning.com/lifesciences](http://www.corning.com/lifesciences)

#### ASIA/PACIFIC

**Australia/New Zealand**  
t 61 427286832

**Chinese Mainland**  
t 86 21 3338 4338  
f 86 21 3338 4300

**India**  
t 91 124 4604000  
f 91 124 4604099

#### Japan

t 81 3-3586 1996  
f 81 3-3586 1291

#### Korea

t 82 2-796-9500  
f 82 2-796-9300

#### Singapore

t 65 6572-9740  
f 65 6735-2913

#### Taiwan

t 886 2-2716-0338  
f 886 2-2516-7500

#### EUROPE

[CEurope@corning.com](mailto:CEurope@corning.com)

#### France

t 0800 916 882  
f 0800 918 636

#### Germany

t 0800 101 1153  
f 0800 101 2427

#### The Netherlands

t 020 655 79 28  
f 020 659 76 73

#### United Kingdom

t 0800 376 8660  
f 0800 279 1117

#### All Other European Countries

t +31 (0) 206 59 60 51  
f +31 (0) 206 59 76 73

#### LATIN AMERICA

[grupoLA@corning.com](mailto:grupoLA@corning.com)

#### Brazil

t 55 (11) 3089-7400

#### Mexico

t (52-81) 8158-8400