PBMC Stimulation with Peptide Pools and Fluorospot Assay

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 We use this protocol and it's working

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PBMC Stimulation with Peptide Pools and Fluorospot Assay

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ABSTRACT

The purpose of this protocol is to stimulate PBMCs with peptide pools for 14 days followed by Fluorospot assay.

ATTACHMENTS

[cpvbbg4xf.pdf](#)

MATERIALS

- Peptide pools
- Cryopreserved PBMC
- Media (complete RPMI with 5% human serum)
- 50 mL tubes
- Water bath
- Centrifuge

Benzonase **Merck MilliporeSigma (Sigma-Aldrich) Catalog #70664-3**

Recombinant Human IL-2 Protein **R&D Systems Catalog #202-IL**

Trypan Blue **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T8154**

SAFETY WARNINGS

For safety wans hazard warnings, please see the Safety Data Sheet (SDS).

Thawing of Cryopreserved PBMC

1 Add 10 mL media (complete RPMI with 5% human serum) and 20 µL Benzonase per vial of cells to a 50 ml tube.

2 Place PBMC vials in 37 °C water bath for approximately 60-90s. 00:01:30

1m 30s

3 Before cells are completely thawed, add them to the prepared 50 ml tube.

4 Centrifuge at 1200 rpm, 00:07:00 .

5 Pour off media and resuspend in media.

6 Count cells and record viability (trypan blue).

7 Centrifuge at 1200 rpm, 00:07:00 .

8 Pour off media and resuspend according to cell count to a final concentration of 4×10^6 cells/ml.

9 Record peptide pool information:

Donor	
Location	
Vials	
Cells/ml ($\times 10^6$)	
Volume (mL)	
Total # PBMCs ($\times 10^6$)	
Volume to reach 4×10^6 /ml (mL)	
% viability	

In vitro Restimulation for 14 days (Plate cells (2×10^6 per we.

10 Resuspend thawed PBMC at 4×10^6 /ml.

11 Add 0.5 mL cells per well and 0.5 mL stimulus ($5 \mu\text{g}/\text{ml}$ final concentration) .

Note

Plate 1	1	2	3	4	5	6
A						
B						
C						
D						

Plate Layout

12 Add media supplemented with 10 U/mL (final concentration) of rIL-2 every 3-4 days.

12.1 **Day 4** – add 1 mL media supplemented with IL-2.

12.2 **Day 7 and 11** – replace 1 mL media supplemented with IL-2 without disturbing cells.

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