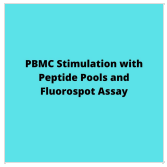

Jul 19, 2021

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

DOI

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PROTOCOL CITATION

cecilia , Alessandro Sette 2021. PBMC Stimulation with Peptide Pools and Fluorospot Assay. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bphjmj4n

KEYWORDS

PBMC, peptide pools, Fluorospot assay

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MATERIALS TEXT

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

Benzonase **Sigma Catalog #70664-3**

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

Trypan Blue **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 $4x10^6$ cells/ml.

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9 Record peptide pool information:

Donor	
Location	
Vials	
Cells/ml (x10^6)	
Volume (mL)	
Total # PBMCs (x10^6)	
Volume to reach 4x10^6 /ml (mL)	
% viability	

In vitro Restimulation for 14 days (Plate cells (2x10^6 per well) in 24-well plate)

10 Resuspend thawed PBMC at 4x10^6/ml.

11 Add **0.5 mL cells** per well and **0.5 mL stimulus (5µg/ml final concentration)** .

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.

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

13

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