Antigen Specific Stimulation of T-Lymphocytes (PBMC)

Important preliminary calculation: To stimulate PBMC, you need at least 1 µg of each peptide per ml of the final test solution. Determine how much peptide solution you need to add to achieve the desired peptide concentration per test.

It is best to use heparinized or citrated blood and to prepare the PBMC by density gradient centrifugation using Ficoll-Paque.

Protocol

After gradient centrifugation, wash cells twice with sterile phosphate-buffered saline (PBS).

Resuspend cells in supplemented 1640 RPMI medium to achieve a concentration of 1.25 to 2.5 x 10^6 cells/ml.

Supplement 1640 RPMI medium with:
- 2 mmol/l L-glutamine
- 10% (v/v) heat-inactivated fetal bovine serum
- 100 I.U. Penicillin or streptomycin.

Add a sample volume of the peptide solution to supplemented 1640 RPMI medium for a total volume of 100 µl.

Pipette 100 µl of the peptide-medium mixture into sterile 5 ml Falcon tubes.

Polypropylene tubes are best because cells normally do not adhere to them. This way you can avoid using EDTA to detach cells.

Plan tubes with controls:
- Positive control: 100 µl of supplemented medium with CEF Control Peptide Pool advanced
- Negative control: 100 µl of supplemented medium with HUMAN (Actin) Peptide Pool
- Unstimulated control: 100 µl of supplemented medium

Note: If you used DMSO to dissolve the peptides to be tested, use the same amount of DMSO in your controls.

Add 400 µl of the cell suspension to each tube.

Place the tubes in a rack at a 5° slant – so the tubes are almost lying.

Incubate the tubes in this position at 37°C in a H_2O-saturated, 5% CO_2 atmosphere.

After two hours, pipette 500 µl of supplemented medium containing 10 µg of Brefeldin A into each tube.

Put the rack back into the incubator.

Note: To ensure that all cells are securely covered with medium, the tubes must be at exactly the same tilt as before.

After another 4 hours, pipette 3 ml of cold PBS into each tube.

Centrifuge: 8 min, 430 x g, 4 °C, and decant or aspirate the supernatant.

Resuspend the cell pellets in the remaining liquid.

Add 3 ml of PBS containing 2 mM EDTA.

Note: The area of the tube wall where cells could have adhered must be covered with EDTA buffer.

Incubate the tubes for 10 minutes at 37°C in a water bath.

Vortex the tubes for 30 seconds at low speed.

Centrifuge: 8 min, 430 x g, 4°C, and decant or aspirate the supernatant.

Resuspend the cell pellets in the remaining liquid.

Add 1 ml of PBS containing 0.5% (w/v) bovine serum albumin and 0.1% (w/v) sodium azide.

Centrifuge: 8 min, 430 x g, 4°C, and decant or aspirate the supernatant.

Resuspend the cell pellets in the remaining liquid.

Now you can stain the surfaces, permeabilize the cells and stain intracellularly. Use your usual protocol or the manufacturer's standard protocols.