Innovative Peptide Solutions

Protocol

PepMix™ Collection

for Antigen Specific Stimulation of T-Lymphocytes

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I. Protocol for dissolving freeze dried PepMixes™

The PepMix™ Collection contains 2 x 1µg (approx. 0.6 nmol) of each of the peptides spanning the corresponding antigenic proteins.

Peptides are 15meres with an overlap of 11 amino acids that were chemically synthesized, purified and analyzed by LC-MS.

To dissolve the PepMix™ Collection DMSO can be added to the freeze dried material in fixed aliquotes of volume until the peptides are completely dissolved (we suggest to do the calculations before you start). According to our experience, approximately 2 µl of DMSO are sufficient to dissolve the material of one vial. Vortexing and sonication may help to accelerate the dissolution process.

The dissolved mix can be stored at -20°C or diluted with an appropriate buffer system, e.g. phosphate-buffered saline (PBS) for immediate use in cell stimulation and intracellular cytokine detection (see subsequent section).

Please avoid repeated thawing/freezing cycles of stored aliquots.

Please note that the final concentration of DMSO must be below 1 % (v/v) to avoid toxicity in the biological system.

Calculate a minimum of 1 µg/ml final concentration of each peptide per test for stimulating PBMC. For whole blood (not recommended) higher concentrations are generally required.

Determine the resulting volume of peptide solution to be added per tests to achieve the desired peptide concentration.

II. Protocol for stimulation and intracellular cytokine detection

We recommend the use of heparinized or citrated blood and PBMC that are prepared by density gradient centrifugation using Ficoll-Paque. This protocol is for PBMC and alterations to this protocol may be necessary when using different materials such as whole blood, joint fluid aspirates, etc.

After gradient centrifugation, wash cells twice with sterile phosphate-buffered saline (PBS) and resuspend in “supplemented“ 1640 RPMI media containing 2 mmol/l L-Glutamine, 10% (v/v) heat-inactivated fetal calf serum, and 100 I.U. Penicillin or Streptomycin (antibiotics may not be necessary for short term stimulation).

- Pipette 100 µl of supplemented media containing one test volume of peptide solution into sterile 5 ml tubes (e.g. Falcon 2054). Place 100 µl of supplemented media containing a corresponding amount of DMSO in one tube, which will serve as unstimulated control. Other tubes or culture dishes may be used as well. The use of polypropylene tubes normally prevents cell adhesion to the tubes. Thus, the use of EDTA (see below) to detach cells can be omitted.
- Add 400 µl of cell suspension (containing 500,000 to 1,000,000 cells, i.e. 1.25 to 2.5 x 10^6 cells /ml) to each tube.
- Place tubes in a rack and place the rack in a standard incubator (37°C, H_2O-saturated 5% CO_2-atmosphere) at a 5° slant (the tubes are almost lying horizontally).
- After two hours, add 500 µl of supplemented media containing 10 µg of Brefeldin A to each tube.
- Put rack back into the incubator observing that each tube must be in the very same (slanted) position as before. Otherwise adherent cells may not be covered by media.
- After 4 additional hours (6 hours in total) add 3 ml of cold PBS to each tube.
- Centrifuge (430 x g, 8 min, 4°C) and decant or aspirate supernatant.

- Resuspend the cell pellets in the remaining fluid.
- Add 3 ml of PBS containing 2 mM EDTA. Assure that the area of the tube wall which may have adherent cells on it, is indeed covered with EDTA-buffer.
- Incubate all tubes for 10 min at 37°C (water bath).
- Vortex tubes at a low speed for 30 seconds.
- Centrifuge again (430 x g, 8 min, 4°C) and decant or aspirate supernatant.
- Resuspend the cell pellets in the remaining fluid.
- Add 1 ml of PBS containing 0.5% (w/v) bovine serum albumin and 0.1% (w/v) sodium azide.
- Centrifuge (430 x g, 8 min, 4°C) and decant or aspirate supernatant
- Resuspend pellets in remaining fluid.
- Proceed with surface staining, cell permeabilization and intracellular staining according to your usual protocol or refer to the manufacturer's standard protocols.

Flow cytometric analysis and interpretation

During data acquisition live gates that exclude potentially reactive cells must be avoided. Make sure that lymphocyte live gates are not too small. Avoid other live gates. Read as many events as possible to increase the quality of your analysis.