

User Manual

PepSup Kit

A ready-to-use highly standardized Peptide-Based ex-vivo Stimulation Assay for Whole-Blood Samples

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Please read the entire Manual before starting your Experiments!
Carefully note the handling and storage conditions.
For research use only. Not intended for any animal or human therapeutic or diagnostic use.

1. 1. Introduction

PepSup™ is a highly standardized, peptide-based cell stimulation assay. It was designed to produce short-term culture supernatants that can subsequently be analyzed with respect to cytokines or other mediators of interest. Whereas PepSup™ was designed for use with whole blood, it may also be used with other cell suspensions. Sample processing requires a programmable heat block and a simple bench-top centrifuge. Following the stimulation protocol, samples are centrifuged and supernatants collected. These can be analyzed immediately or frozen for later analysis.

PepSup™ is the ideal basis for monitoring antigen-specific, stimulation-induced mediator secretion in a range of situations, for example in the study of infection, transplantation, or tumour-specific immunity. Because of its high degree of standardization and hence reproducibility, it is particularly well-suited to longitudinal monitoring.

1.1 Components of the Kit

The PepSup™ kit contains 12 tubes with specific reagents, 12 positive control tubes, and 12 negative control tubes as indicated on the kit and tube labels. We recommend that all tubes are run in duplicate, in which case the kit is sufficient for 6 stimulations with the pertinent controls.

Tube A (red tops) contains specific stimulatory peptides in PepSup™ culture media.

Tube B (green tops) contains the positive control in PepSup™ culture media.

Tube C (transparent tops) contains the negative control in PepSup™ culture media.

PepSup™ media is intended for use with closed tubes in a heat block or other incubator (see below).

1.2 Storage & Handling

The PepSup™ Kit is shipped on dry ice and should be stored at -80°C. Tubes should be removed from the freezer and stored at room temperature for 1 hour (or until temperature has equalized) before use.

All kit reagents are stable for a minimum of 6 months from delivery.

2. Experimental Protocol



We recommend running technical duplicates for each processed sample.

1. Remove the required number of tubes (A, B, and C) from freezer. Allow 1 h to defrost at room temperature.
2. Set the programmable heat-block to the desired incubation temperature and time (for example 37°C, 16 hours or as required). If desired and supported by your device, the incubation can be stopped automatically after the desired incubation time by cooling samples to 4°C. This can be helpful if the incubation time ends outside working hours, allowing sample processing to continue the next morning.
Note: If no heat block is available a dry bath, incubator or equivalent providing an ambient temperature of 37°C can be used alternatively but tubes need to be closed tightly.
3. Add 500 µL of heparin-anticoagulated whole blood to each tube, close tubes and then invert gently to mix contents. Place tubes in the heat block and begin incubation.
Alternatively, add 500 µL of cell suspension containing 1x10⁶ PBMC in a CO₂ independent cell culture medium (e.g. in CO₂ Independent Medium, ThermoFisher Scientific, Ordering Number: 18045088).
Note: If you proceed as instructed, the final assay volume is 1 mL and the final concentration of each individual peptide in the stimulation assay is 2 µg/mL.
4. At the end of the desired incubation time, remove tubes from heat block, and centrifuge for 8 min at 2000 x g.
5. Carefully collect supernatants without disturbing the cell pellet and analyze immediately or store frozen (-20°C or colder) until analysis.

To ascertain reproducibility, you should use a standardized protocol for measuring mediators in the supernatant.

3. Contact Us

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4. Product Use & Liability

THE PRODUCTS ARE FOR EXPERIMENTAL LABORATORY USE ONLY AND NOT INTENDED FOR HUMAN OR HOUSEHOLD USE.

Only qualified personnel should handle these chemicals.

Note that missing hazard warnings do not indicate that a product is harmless. Products are for research use only (RUO). JPT Peptide Technologies declines responsibility for any damage arising from the inappropriate use of its products.

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