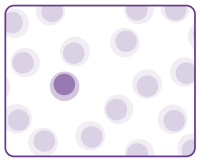
Short protocol

Cytokine Secretion Assay – Detection Kit

For details on the procedure and analysis refer to protocol supplied with the kit.

A In vitro restimulation of the cells



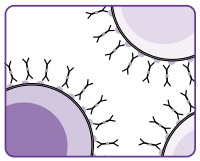
1. Prepare **10^6** cells per sample and wash cells by adding medium, centrifuge at 300xg for 10 minutes.
2. Resuspend 10^6 cells per sample in 100 µl culture medium, containing 5% human or mouse serum.

   Transfer cells in one well of a 96-well-plate per sample.
3. Add respective antigen or control reagent and incubate at 37°C, 5-7% CO_2:

   peptide: e.g. 1-10 µg/ml, 3-6 hours
   protein: e.g. 10 µg/ml, 6-16 hours
   SEB: e.g. 1 µg/ml, 3-16 hours
4. Collect cells carefully by pipetting up and down. Rinse the dish with cold buffer. Check microscopically for any remaining cells, if necessary, rinse the dish again.

For each test with **10^6** total cells, prepare:
50 ml of **cold buffer** (4-8°C),
100 µl of **cold medium** (4-8°C),
1 ml (or **10 ml**; see table below) of **warm medium** (37°C).

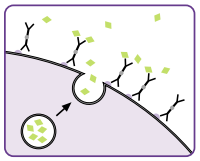
B Labeling cells with Cytokine Catch Reagent



1. Use **10^6** total cells in a 2 or 15 ml closable tube (see table below) per sample.
2. Wash cells by adding **1-2 ml** of **cold buffer**, centrifuge at 300xg for 10 minutes at 4-8°C, pipette off supernatant completely.

   ▲ **Note:** When working with Mouse Cytokine Secretion Assays repeat wash step.
3. Resuspend cell pellet in **90 µl** of **cold medium**.
4. Add **10 µl** of **Cytokine Catch Reagent**, mix well and incubate for **5 minutes on ice**.

C Cytokine secretion period

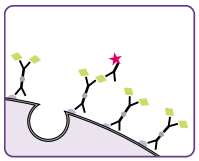


1. Add **warm medium** (37°C) to dilute the cells according to the following table: *

Expected number of secreting cells	Dilution	Amount of medium to add per 10^6 total cells
< 5 %	10^6 cells/ml	1 ml
≥ 5 %	≤ 10^5 cells/ml	10 ml

2. Incubate cells in closed tube for **45 minutes** at **37°C** under slow continuous rotation using the MACSmix, or turn tube every 5 minutes to resuspend settled cells.

D Labeling cells with Cytokine Detection Antibody



1. Put the tube **on ice.**
2. Wash the cells by filling up the tube with **cold buffer**, and centrifuge at 300xg for 10 minutes at 4-8°C. Pipette off supernatant completely.

   ▲ **Note:** If the volume of the cell suspension was higher than the volume of added buffer, repeat wash step.

   ▲ **Note:** When working with Mouse Cytokine Secretion Assays repeat wash step.
3. Resuspend cell pellet in **90 µl** of **cold buffer**.
4. Add **10 µl** of **Cytokine Detection Antibody**.
5. (Optional) Add additional staining reagents.
6. Mix well and incubate for **10 minutes on ice.**
7. Wash cells by adding **2 ml** of **cold buffer,** centrifuge at 300xg for 10 minutes at 4-8°C, pipette off supernatant.
8. Resuspend cells in **500 µl** of **cold buffer,** and proceed to flow cytometric analysis.

   ▲ **Note:** For flow cytometric analysis add propidium iodide or 7-AAD to a final concentration of 0.5 µg/ml just prior to acquisition.

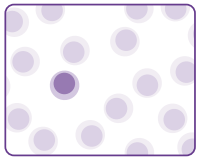
* When working with **Mouse IFN-γ Secretion Assay** and expected frequencies of IFN-γ secreting cells ≥ 2%, refer to protocol supplied with the kit.

Short protocol

Cytokine Secretion Assay – Detection of cytokine secreting cells from whole blood

For details on the procedure and analysis refer to protocol supplied with the kit.

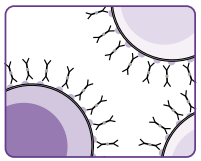
A In vitro restimulation of the cells



1. Start with **250 µl of fresh, sodium heparinized, human whole blood** (containing about $5x10^5$ lymphocytes) in a 15 ml conical tube.
2. Add respective antigen or control reagent and incubate at 37°C:
   peptide: e.g. 1-10 µg/ml, 3-6 hours
   protein: e.g. 10 µg/ml, 6-16 hours
   SEB: e.g. 1 µg/ml, 3-16 hours

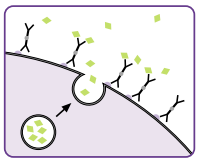
For each test with 250 µl of whole blood, prepare:
50 ml of **cold buffer** (4-8°C),
100 µl of **cold medium** (4-8°C),
5 ml of **warm medium** (37°C),
5 ml of **erythrocyte lysing solution** (room temperature).

B Labeling cells with Cytokine Catch Reagent



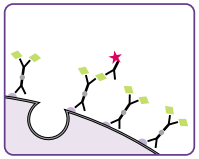
1. Resuspend cells and wash cells by adding **10 ml** of **cold buffer**. Centrifuge at 300xg for 10 minutes at 4-8°C, pipette off supernatant carefully.
   ▲ **Note:** Be careful, leukocytes will appear on top of the loose red pellet.
2. Resuspend cells in **80 µl** of **cold medium**.
3. Add **20 µl** of **Cytokine Catch Reagent**, mix well and incubate for **5 minutes on ice**.

C Cytokine secretion period



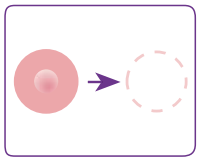
1. Add **5 ml** of **warm medium** (37°C) to dilute the cells.
2. Incubate cells in closed tube for **45 minutes** at **37°C** under slow continuous rotation using the MACSmix, or turn tube every 5 minutes to resuspend settled cells.

D Labeling cells with Cytokine Detection Antibody



1. Put the tube on ice.
2. Wash the cells by adding **10 ml** of **cold buffer**, centrifuge at 300xg for 10 minutes at 4-8°C. Pipette off supernatant completely.
3. Resuspend cells in **80 µl** of **cold buffer.**
4. Add **20 µl** of **Cytokine Detection Antibody.**
5. (Optional) Add additional staining reagents.
6. Mix well and incubate for **10 minutes on ice.**

E Lysis of erythrocytes



1. Add **5 ml** of **erythrocyte lysing solution**. Mix gently and incubate for **10 minutes** at **room temperature.** Rotate tube continuously using the MACSmix, or turn tube several times during incubation time.
2. Centrifuge at 300xg for **10 minutes** at **room temperature**. Pipette off supernatant completely.
3. Wash cells by adding of **10 ml** of **cold buffer,** centrifuge at 300xg for 10 minutes. Pipette off supernatant.
4. Resuspend cells in **500 µl** of **cold buffer**, and proceed to flow cytometric analysis.
   ▲ **Note:** For flow cytometric analysis add propidium iodide or 7-AAD to a final concentration of 0.5 µg/ml just prior to acquisition.

130-091-149