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Antigen Presentation Protocol

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1 Works for me dx.doi.org/10.17504/protocols.io.bpk4mkyw

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ABSTRACT

This protocol details methods for 3-day Antigen Presentation Assay.

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MATERIALS TEXT

REAGENTS

- LPS/IFNg (including controls)
- RPMI media
- PFA
- PBS
- Wash buffer: DMEM with 0.1M glycine + 10% iFBS
- Stock peptide
- 1x lysis buffer (stock = 5x, with triton, pH = 7.8) in dH2O
- 1M DTT
- CPRG buffer
- Water
- CPRG

CONSUMABLES

- 96-well plates
- 50ml conical tubes

EQUIPMENT

- Incubator: 37°C with 5% CO2
- Centrifuge
- Cell counter
- Plate reader

SAFETY WARNINGS

Please refer to Safety Data Sheets (SDS) for health and environmental hazards.

ABSTRACT

This protocol details methods for 3-day Antigen Presentation Assay.

Day 1 1d

1 Prepare a 96 well plate:
- Label appropriately (experimental condition – LPS/IFNg controls, infection controls, etc.)
- Duplicate labelled wells for the peptide control

2 

Scrape RAW cells and resuspend (pipette up and down 8x).

3 Count RAW cells and adjust concentration to **0.75 million cells/ml**.

4 

Add **200 µl RAW cells** to each well.

5 Add LPS/IFNg accordingly.

6  1d

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Incubate for **24:00:00** at **37 °C with 5% CO2** .

Day 2 10m

7 Prior to beginning, check the cell's media (red/orange = good, yellow = bad).

8 Prepare **1 % (v/v) PFA in PBS** at **Room temperature** (pH **7.4**).

9 

Discard supernatant in the 96 well plate and add **50 µl 1% PFA** .

9.1  10m

Incubate at **Room temperature** for **00:10:00** .

10 Prepare 2E2 cells while RAW cells are in 1% PFA:

10.1 

Pour flask into 50 ml conical and centrifuge at **1500 rpm, 00:03:00** .

To keep more 2E2 cells growing, add **50 mL RPMI media** back into their flask and incubate at **37 °C , 5% CO2** .

10.2 Count 2E2 cells and adjust concentration to **0.4 million cells/ml**.

11 

Add **200 µl wash buffer** to each well in the 96 well plate and discard immediately.

Wash buffer = DMEM with 0.1M glycine, + 10% iFBS.

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11.1

Repeat 2x more: Add **200 µl wash buffer** to each well in the 96 well plate and discard immediately. **(Wash 1/2)**

11.2

Repeat 1x more: Add **200 µl wash buffer** to each well in the 96 well plate and discard immediately. **(Wash 2/2)**

12

Add **250 µl 2E2 cells** per well in ½ of the samples (the non-peptide ones). Add an extra row of 2E2 cells alone for a negative control.

13 Dilute stock peptide (1 µg/ml) **5000 fold** in the 2E2 cells (final concentration: **0.2 nanogram per milliliter (ng/mL)**).

14

Add **250 µl peptide + 2E2 cell solution** to the remaining wells.

15 16h

Incubate at **37 °C , 5% CO2** for **16:00:00 (NO LONGER)** .

Day 3 5m

16 Prepare 1x lysis buffer (stock = 5x, with triton, pH **7.8**) in dH_2O.

16.1

Add **30 µl 1M stock of DTT** in **10 mL lysis buffer** .

17 Prepare CPRG buffer (pH **7.8**).

18

Centrifuge 96 well plate at **2200 rpm, 00:01:00** .

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19

Add **50 µl lysis buffer** to all wells.

19.1 5m

Incubate at **Room temperature** for **00:05:00** (up to 20 minutes MAX).

20

Prepare CPRG (recipe below = for each well)

- **150 µl CPRG buffer**
- **20.2 µl water**
- **0.046 mg CPRG**

21

When lysis is done, add **170 µl CPRG solution** / well.

21.1

Incubate either at **Room temperature** or 37°C (37 speeds up reaction to about ~ 20 minutes for peptide samples).

22

Transfer **150 µl colored solution** to a new plate.

Take care not to transfer debris or make bubbles.

23

Take reading at 595 nm or 570 nm.

* To stop reaction to leave overnight, incubate at **4 °C** .

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