Cell Surface Mild Acid Elution of MHC-bound Immunopeptides

Teesha C Luehr

1University of British Columbia

Leonard Foster's Lab

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Protocol status: Working
We use this protocol and it's working

Created: Mar 13, 2019
1 Culture cells. If culturing THP-1s, follow the protocol.

1.1 The base medium for this cell line is RPMI-1640

1.2 Required supplements:
   - [M] 1% volume L-glutamine
   - [M] 10% volume Fetal Bovine Serum

   Note
   Most catalog numbers of RPMI-1640 contain L-glutamine, however, some do not. Ensure that it is in the media before using it for culturing.

1.3 Optional Supplements:
   - [M] 1% volume PenStrep
   - [M] 0.05 millimolar (mM) 2-mercaptoethanol

   Note
   PenStrep is not required for THP-1 culturing, however, if you are having issues with bacterial contamination, it can be used at 1X.
2-mercaptoethanol is stated as a required component for complete RPMI-1640 medium, however, in our laboratory it is not standard practice to add it.

1.4 Always store cells in liquid nitrogen. This is for both the original tube of cells from ATCC and any passages afterwards.

1.5 Place the media bottle in the 37 °C water bath at least 00:30:00 prior to using.

1.6 Thaw cells at 25 °C (room temperature) for 00:10:00 or 37 °C in a water bath for 00:02:00.

1.7 Sanitize all items going into the Biological Safety Cabinet with 70% ethanol.

1.8 As soon as the cells are thawed, transfer the cells to a 15 mL conical tube and add 10 mL of complete media.

Note

Cells are stored with 5% DMSO, which can lyse cells if they are left for too long.

1.9 Pellet cells for 00:03:00 at 500g.
1.10 Discard supernatant

1.11 Resuspend cells by pipetting up and down 5X in 5 mL complete media

1.12 Transfer cells + media to a T-25 flask

1.13 Incubate cells at 37 °C and 5% CO2 and 80% humidity

1.14 THP-1 cells replicate after ~26 hours. In practice, it takes 2 days for a true doubling.

1.15 Once cells have doubled OR when media has begun to change colour, it is time to add media, split cells into new flasks, or to spin down to remove all media

Step 1.15 includes a Step case.

Adding media
Splitting cells into new flasks
Spinning cells down to remove all media

Adding media

1.16 If concerned about cell concentration, perform a cell count

1.17 Double the total media volume with new complete media
1.18 Carefully mix the new media in by rocking the flask back and forth

1.19 Place the flask back in the incubator

2 Grow cells to a total number of 1-2x10^8 cells

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**Preparing Reagents**

3 1X PBS at 25 °C (room temperature)
   - 8 g NaCl
   - 0.2 g KCl
   - 1.44 g Na2HPO4
   - 0.24 g KH2PO4
   - 1 L Milli-Q water
   - pH adjust to 7.4 with 37% HCl

4 1X PBS at 4 °C
   
   **Note**
   
   This PBS solution does NOT have to be sterile, but make sure it is clearly marked.

5 1X saline (PBS without the phosphate) at 4 °C
   - 4 g NaCl
   - 0.1 g KCl
   - 500 mL Milli-Q water
   - no need to pH adjust, it's not a buffer

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no need to sterilize as the mild acid elution is not done under sterile conditions

6 1X saline + 2% acetic acid at 4 °C
   - 245 mL of above 1X saline solution
   - 5 mL acetic acid

7 Each 250 mL T-175 flask will require 7 50 mL conical tubes

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**Pelleting & Rinsing Cells**

8 Cells will most likely be in 250 mL complete media. Use five 50 mL conical tubes to pellet all cells from one flask at a time.

9 Pellet cells for 00:03:00 at 500g

10 Discard supernatant

11 Resuspend each pellet by pipetting up and down 5X with 5 mL room temp PBS (sterile in the hood)

12 Combine all five conical tubes into one

13 If cell count is not know, perform one now to confirm a minimum of 1x10^8 cells
14  Pellet cells for 00:03:00 at 500g

15  Discard supernatant

16  Resuspend each pellet by pipetting up and down 5X with 10 mL cold PBS

17  Pellet cells for 00:03:00 at 500g

18  Discard supernatant

19  Resuspend each pellet by pipetting up and down 5X with 10 mL cold PBS

20  Pellet cells for 00:03:00 at 500g

21  Discard supernatant
22. Resuspend each pellet by pipetting up and down 5X with 10 mL cold saline.

23. Transfer cells + saline to a new 50 mL conical tube.

**Note**
This is to remove any large amounts of phosphate.

24. Pellet cells for 00:03:00 at 500g.

25. Discard supernatant.

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**Mild Acid Elution**

26. Resuspend each pellet by pipetting up and down 5X with 10 mL cold 2% acetic acid in saline.

**Note**
EXTREMELY IMPORTANT, REMEMBER THAT THE PEPTIDES WILL BE IN THE SUPERNATANT.

27. DO NOT DISCARD SUPERNATANT.
Collect the supernatant in a new 50 mL conical tube.

Freeze supernatant at -80 °C overnight.

Lyophilize sample for 48 hours.

Peptides are now ready to be desalted with chosen clean up method (STAGE tip, LC-UV, combination, etc).