Immunocytochemistry HeLa Cells

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Abstract

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Protocol

Step 1.

Place one autoclaved coverslip into each well of a six well plate. If an autoclave is not available, sterilization can be performed by dipping coverslips into 95% Ethanol. After dipping, use open flame to burn off ethanol.

Step 2.

Coat coverslips with PDL and Laminin (30ml 1X PBs, PDL, Laminin - mix by inversion) - add 1.5ml to each well of a six well plate.

Step 3.

Rock gently to coat coverslips. Be sure there are no bubbles underneath the coverslips.

Step 4.

Allow coverslips with PDL and Laminin to incubate in tissue culture incubator for a minimum of 1-3 hours or a maximum of overnight.

DURATION
03:00:00

Step 5.

Remove PDL and Laminin solution.

Step 6.

Wash with 2ml of PB S for 5 mins

DURATION

00:05:00

Step 7.

Wash with 2ml of PB S for 5 mins

DURATION
00:05:00

Step 8.

Remove PBS and add diluted cells.

Ensure that concentration of cells will allow for confluency within experimental window.

Press down on each coverslip to ensure there are no bubbles underneath.

Step 9.

Incubate cells until the reach the appropriate density for experimental design.

Step 10.

When density is appropriate to begin fixation, remove cell culture media and add 1ml of 4% paraformaldehyde to each well. Incubate for 10 minutes.

DURATION
00:10:00

Step 11.

Remove paraformaldehyde and rinse with 2ml 1X PBS for 5 minutes

DURATION
00:05:00

Step 12.

Remove PBS and rinse a second time with 2ml 1X PBS for 5 minutes.

DURATION
00:05:00

Step 13.

Remove PBS and rinse a third time with 1X 2ml PBS for 5 minutes.

DURATION
00:05:00

Step 14.

Add 1ml blocking solution to each well. Incubate for 15 minutes.

DURATION
00:15:00

Step 15.

Remove blocking solution, rinse once with 2ml of 1X PBS for 5 minutes.

DURATION
00:05:00

Step 16.

Remove PBS and add 1ml of blocking solution to each well. Dilute primarantibodies into blocking solution as appropriate.

See list here:
https://www.protocols.io/view/Optimized-Concentrations-for-Developmental-Studies-fewbjfe

Incubate a minimum of 2hrs at room temp or overnight at 4 degrees Celsius.

DURATION
02:00:00

Step 17.

Remove block with primary antibodies and rinse with 2ml of 1X PBS for 5 minutes.

DURATION
00:05:00

Step 18.

Remove 1X PBS and rinse again with 2ml of 1X PBS for 5 minutes

DURATION
00:05:00

Step 19.

Remove 1X PBS and rinse again with 2ml of 1X PBS for 5 minutes

DURATION
00:05:00

Step 20.

Remove 1X PBS and add 1ml blocking solution to each well. Incubate for 15 minutes.

DURATION
00:15:00

Step 21.

Remove blocking solution and rinse once with 1X PBS for 5 minutes.

Step 22.

Remove PBS and add 1ml of blocking solution to each well. Dilute secondary antibodies into blocking solution as appropriate.

I use 1ul per ml of donkey antibodies that have reconstituted to the manufactures specifications. This is also the time that you can add DAPI or Hoescht, if desired.

Incubate for 2 hours wrapped in foil.

DURATION
02:00:00
Step 23.

Remove block with secondary antibodies.

Rinse with 2ml of 1X PBS for 5 minutes

DURATION
00:05:00
Step 24.

Remove 1X PBS and rinse again with 2ml of 1X PBS for 5 minutes

DURATION
00:05:00
Step 25.

Remove 1X PBS and rinse again with 2ml of 1X PBS for 5 minutes

DURATION
00:05:00
Step 26.

Remove 1X PBS and add 1ml blocking solution to each well. Incubate for 15 minutes.

DURATION
00:15:00
Step 27.

Remove blocking solution and rinse once with 2ml of 1X PBS.

Step 28.

Mount slides using mounting media.

Store at -20 degrees celsius.