Immunofluorescence Detection of Progesterone Receptor in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
1X Citrate Buffer
ProLong Gold Mounting Media

Blocking Serum: Normal Goat Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 005-000-121

Primary Antibody: Mouse Anti-Progesterone Receptor Antibody
Beckman Coulter, Inc.
Fullerton, CA 92834
www.beckmancoulter.com
1-800-458-5060
Catalog # IM1546

Negative Control Serum: Purified Mouse IgG2a Isotype Control Serum
BD Biosciences
San Jose, CA 95131
www.bdbiosciences.com
1-855-236-2772
Catalog # 550339

Secondary Antibody: Alexa Fluor® 488 Goat Anti-Mouse IgG (H+L) *****Highly Cross-Adsorbed*****
Life Technologies / Invitrogen
Grand Island, NY 14072
www.invitrogen.com
1-888-584-8929
Catalog # A-11209

Staining Procedure

Positive Control Tissue: Female reproductive tract
Stain localization: Nuclear

1. Deparaffinize and hydrate slides through the following solutions:

Solution	Repetitions	Time
Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X wash buffer	2 times	5 minutes

2. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.

3. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

4. Heat-Induced Epitope Retrieval Using The Decloaker
   Add 500 ml of distilled water to the pan inside the decloaker.
   Place a full rack of slides into a Tissue Tek® container with 200 ml of 1X citrate buffer
   (Insert blank slides into any empty slots in the rack to ensure even heating of slides)
   Place the container stably inside the pan and decloak for 5 minutes. *Maximum Pressure* _____
   Depressurize for 10 minutes.
   Remove pan top and cool for 10 minutes. *Temperature Before Cooling Slides*_____
   Rinse the slides in 2 changes of distilled water for 3 minutes each time.

5. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each time.

6. Block with 10% normal goat serum for 20 minutes at room temperature.
   Lot #_____ Date Reconstituted_____

   DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.
   ONLY WIPE EXCESS BUFFER.

7. Apply primary antibody at a 1:150 dilution. Incubate for 1 hour at room temperature.
   Lot #_____ Exp. Date _____

   For negative control slides, dilute mouse IgG2a control serum so that it's IgG2a protein concentration matches that of the primary antibody (if necessary). Then make a 1:150 dilution. If the concentrations can't be matched using this method, the dilution for the negative reagent may need to be adjusted. Apply the negative and incubate for 1 hour at room temperature.
   Lot #_____ Exp. Date _____

8. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

From this point on, please perform the stain in the dark.

9. Apply the Alexa 488 goat anti-mouse secondary antibody at a 1:300 dilution. Incubate for 1 hour at room temperature.
   Lot #_____ Exp. Date _____

10. Rinse the slides in 2 changes of 1X Wash buffer for 5 minutes each.

11. Rinse the slides in tap water.

12. Coverslip with ProLong Gold Mounting Media (with or without DAPI).

13. Store slides at 4°C

Updated 06/2010