Novocastra™ Liquid Mouse Monoclonal Antibody Progesterone Receptor

Product Code: NCL-L-PGR

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Human progesterone receptor.
Clone	1A6
Ig Class	IgG1
Antigen Used for Immunizations	Synthetic peptide corresponding to a site of predicted high antigenicity on the human progesterone receptor.
Hybridoma Partner	Mouse myeloma (p3-NS1-Ag4-1).
Preparation	Liquid tissue culture supernatant containing 15 mM sodium azide. Volume as indicated on vial label.
Effective on Frozen Tissue	Yes. Optimum fixative, Zamboni's, 10 minutes at 25 °C (see Stefanini et al., 1967).
Effective on Paraffin Wax Embedded Tissue	Yes (using the high temperature antigen unmasking technique: see overleaf).
Recommendations on Use	Immunohistochemistry: Typical working dilution for frozen sections 1:40. 60 minutes primary antibody incubation at 25 °C. Indirect technique. NCL-GAMP is the recommended secondary antibody. Technique is critical to achieve good results with NCL-L-PGR (see overleaf). Typical working dilution for paraffin sections 1:40. High temperature antigen unmasking technique. 60 minutes primary antibody incubation at 25 °C. Standard ABC technique. Western Blotting: Typical working dilution 1:25–1:50.
Positive Controls	Immunohistochemistry: Endometrium. Western Blotting: T47D cell line.
Staining Pattern	Nuclear
Storage and Stability	Store liquid antibody at 4 °C. Under these conditions, there is no significant loss in product performance up to the expiry date indicated on the vial label. Prepare working dilutions on the day of use.
General Overview	The human progesterone receptor (PR) is expressed as two isoforms, PRA (94 kD) and PRB (114 kD), which function as ligand-activated transcription factors. These two isoforms are transcribed from distinct estrogen receptor (ER)-inducible promoters within a single copy PR gene. The PRA form is a truncated version of the PRB form, lacking the first 164 N-terminal amino acids. In humans, PRA acts as a transdominant repressor of the transcriptional activity of PRB, glucocorticoid receptor, ER, androgen receptor and mineralocorticoid receptor. PRB functions mainly as a transcriptional activator. PRB is expressed strongly in endometrial glandular and stromal nuclei in the proliferative phase of the menstrual cycle and weakly during the secretory phase and early pregnancy.

General References

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Instructions for Use

Protocol For Immunohistochemical Detection Of Estrogen And Progesterone Receptors On Frozen Sections

1. Cut 7 micron sections of frozen tissue and fix immediately in Zamboni's fixative for 10 min.
2. Wash 3 x 10 min in TBS.
3. Cover with NGS for 10 min.
4. Remove excess serum, cover with primary antibody and incubate for 60 minutes at 25 °C.
5. Wash in TBS for 2 x 5 min.
6. Cover with secondary antibody and incubate for 30 min at 25 °C.
7. Wash in TBS for 2 x 5 min.
8. Develop with DAB.
9. It is suggested that a counterstain is not used.
10. Dehydrate, clear and mount.

Notes

NGS	Normal goat serum diluted 1 in 5 with TBS
Primary antibody	Diluted with NGS
Secondary antibody	Goat anti-mouse peroxidase conjugated immunoglobulins
TBS	Tris buffered saline, pH 7.6

Zamboni's Fixative (paraformaldehyde/picric acid) Suggested Preparation Method

1. Mix 20 g paraformaldehyde with 150 ml double-filtered, saturated aqueous picric acid.
2. Heat to 60 °C in fume cupboard.
3. Add 2.52 per cent sodium hydroxide in water, drop by drop, until solution is clear.
4. Filter solution and allow to cool.
5. Make up to 1000 ml with phosphate buffer.
   (3.31 g NaH_2PO_4. H_20: 33.77 g Na_2HPO_4. $7H_2O$: 1000 ml distilled water).
   This fixative is stable at room temperature for 12 months.

References

Zamboni's fixative - Nature 216: 174-175 (1967).

Instructions for Use

High Temperature Antigen Unmasking Technique for Immunohistochemical Demonstration on Paraffin Sections

1. Cut and mount sections on slides coated with a suitable tissue adhesive.
2. Deparaffinize sections and rehydrate to distilled water.
3. Place sections in 0.5% hydrogen peroxide/methanol for 10 minutes (or use other appropriate endogenous peroxidase blocking procedure). Wash sections in tap water.
4. Heat 1500 mL of the recommended unmasking solution (0.01 M citrate buffer, pH 6.0 (or Epitope Retrieval Solution, RE7113) unless otherwise indicated overleaf) until boiling in a stainless steel pressure cooker. Cover but do not lock lid.
5. Position slides into metal staining racks (do not place slides close together as uneven staining may occur) and lower into pressure cooker ensuring slides are completely immersed in unmasking solution. Lock lid.
6. When the pressure cooker reaches operating temperature and pressure (after about 5 minutes) start a timer for 1 minute (unless otherwise indicated on the data sheet).
7. When the timer rings, remove pressure cooker from heat source and run under cold water with lid on. DO NOT OPEN LID UNTIL THE INDICATORS SHOW THAT PRESSURE HAS BEEN RELEASED. Open lid, remove slides and place immediately into a bath of tap water.
8. Wash sections in TBS* buffer (pH 7.6) for 1 x 5 minutes.
9. Place sections in diluted normal serum (or RTU Normal Horse Serum) for 10 minutes.
10. Incubate sections with primary antibody. Use Antibody Diluent RE7133 (where available).
11. Wash in TBS buffer for 2 x 5 minutes.
12. Incubate sections in an appropriate biotinylated secondary antibody.
13. Wash in TBS buffer for 2 x 5 minutes.
14. Incubate slides in ABC reagent (or RTU streptavidin/peroxidase complex).
15. Wash in TBS buffer for 2 x 5 minutes.
16. Incubate slides in DAB or other suitable peroxidase substrate.
17. Wash thoroughly in running tap water.
18. Counterstain with hematoxylin (if required), dehydrate and mount.

Solutions

0.01 M CITRATE BUFFER (pH 6.0) or RE7113 (where available).
Add 3.84 g of citric acid (anhydrous) to 1.8 L of distilled water. Adjust to pH 6.0 using concentrated NaOH. Make up to 2 L with distilled water.

1 mM EDTA (pH 8.0) or RE7116 (where available).
Add 0.37 g of EDTA (SIGMA product code E-5134) to 1 litre of distilled water. Adjust pH to 8.0 using 1.0 M NaOH.

20 mM TRIS/ 0.65 mM EDTA/ 0.005% TWEEN (pH 9.0) or RE7119 (where available).
Dissolve 14.4 g Tris (BDH product code 271197K) and 1.44 g EDTA (SIGMA product code E-5134) to 0.55 L of distilled water. Adjust pH to 9.0 with 1 M HCl and add 0.3 mL Tween 20 (SIGMA product code P-1379). Make up to 0.6 L with distilled water. This is a 10x concentrate which should be diluted with distilled water as required (eg 150 mL diluted with 1350 mL of distilled water).

** In most applications, 10 mM phosphate, 0.15 M NaCl, pH 7.6 (PBS) can be used instead of 50 mM Tris, 0.15 M NaCl, pH 7.6 (TBS).*

Safety Note

To ensure the correct and safe use of your pressure cooker, PLEASE READ MANUFACTURER'S INSTRUCTIONS.