

Novocastra™ Lyophilized Rabbit Polyclonal Antibody Human Chorionic Gonadotrophin (beta)

Product Code: NCL-HCGp

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Human chorionic gonadotrophin (beta). NCL-HCGp crossreacts weakly with luteinising hormone.
Antigen Used for Immunizations	The isolated beta-chain of human chorionic gonadotrophin.
Preparation	Lyophilized immunoglobulin fraction purified from rabbit serum diluted in PBS with 1% BSA containing 15 mM sodium azide. Reconstitute with the volume of sterile distilled water indicated on the vial label.
Effective on Frozen Tissue	Yes
Effective on Paraffin Wax Embedded Tissue	Yes
Recommendations on Use	Immunohistochemistry: Typical working dilution 1:500–1:1000. Trypsin digestion of paraffin sections is recommended. 60 minutes primary antibody incubation at 25 °C. Standard ABC technique. Western Blotting: Not evaluated.
Positive Controls	Immunohistochemistry: Placenta
Staining Pattern	Cytoplasmic.
Storage and Stability	Store unopened lyophilized 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. The reconstituted antibody is stable for at least two months when stored at 4 °C. For long term storage, it is recommended that aliquots of the antibody are frozen at -20 °C (frost-free freezers are not recommended). Repeated freezing and thawing must be avoided. Prepare working dilutions on the day of use.
General Overview	Human chorionic gonadotrophin (hCG) is a glycoprotein hormone produced by trophoblastic cells of the placenta beginning 10 to 12 days after conception. Maintenance of the fetus in the first trimester of pregnancy requires the production of hCG, which binds to the corpus luteum of the ovary which is stimulated to produce progesterone which in turn maintains the secretory endometrium. hCG is composed of two subunits, alpha and beta. The alpha subunit of hCG is identical to the subunit of luteinising hormone, thyroid stimulating hormone and follicle stimulating hormone. The common alpha chain and the hormone-specific beta chains have molecular weights of 14 kD and 17 kD, respectively. The hCG beta-subunit is unique in the family of beta-containing glycoprotein hormones in that it contains an extension of 29 amino acids at its COOH end. It is believed that the C-terminal region of the CG-beta subunit plays a role in the intracellular behaviour of the heterodimer.
General References	Muyan M, Furuhashi M, Sugahara T, et al.. Molecular Endocrinology. 10 (12): 1678–1687 (1996). Morrish D W, Marusyk H and Siy O. Journal of Histochemistry and Cytochemistry. 35: 93–101 (1987). Mehta H C and MacDonald D J. Clinica Chimica Acta. 121: 245–250 (1982).



Instructions for Use

Trypsin Digestion for Immunohistochemical Demonstration on Paraffin Sections

1. Preheat the following to 37 °C using a water bath:
 - (i) 200 mL of TBS
 - (ii) 200 mL of distilled water.
2. Dissolve 0.2 g Trypsin 250 and 0.2 g Calcium chloride in the 200 mL of TBS.
3. Once the Trypsin solution is at 37 °C, pH to 7.8 with 1 M sodium hydroxide.
4. Place rehydrated paraffin sections in the distilled water to preheat the sections to 37 °C for a minimum of 5 minutes.
5. Incubate sections in Trypsin solution at 37 °C. The time required will depend on the antibody and tissue, however, 30 minutes is usually sufficient.
6. Rinse sections in running tap water.
7. Proceed with immunohistochemistry protocol.

Reagents Required but not Supplied

50 mM Tris-buffered saline

Trypsin 250: Difco order code 0152-13 (available from Becton Dickinson).

Calcium chloride

1 M Sodium Hydroxide

** Trypsin containing chymotrypsin should always be used. The enzyme activities can vary from a supplier and between batches. Such variations may affect the incubation time required.*