Novocastra™ Lyophilized Rabbit Polyclonal Antibody Immunoglobulin A

Product Code: NCL-IgAp

Intended Use	**For In Vitro Diagnostic Use:** This product is intended for qualitative immunohistochemistry with normal and neoplastic formalin-fixed, paraffin-embedded tissue sections, to be viewed by light microscopy.
Specificity	Alpha-chains of human IgA. NCL-IgAp has been solid-phase absorbed to remove cross-reactivity.
Antigen Used for Immunizations	IgA isolated from a pool of normal human sera.
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	No
Effective on Paraffin Wax Embedded Tissue	Yes
Recommendations on Use	Immunohistochemistry: Typical working dilution 1:100–1:200. Trypsin digestion of paraffin sections is recommended. 60 minutes primary antibody incubation at 25 °C. Standard ABC technique. Western Blotting: Typical working dilution 1:500.
Positive Controls	Immunohistochemistry: Tonsil. Western Blotting: Tonsil.
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	The basic structure of immunoglobulin (Ig) molecules is a tetramer of two light chains and two heavy chains linked by disulfide bonds. There are two types of light chains, kappa and lambda, each composed of a constant domain (CL) and a variable domain (VL). There are five types of heavy chains: alpha, delta, epsilon, gamma and mu, all consisting of a variable domain (VH) and three (in alpha, delta and gamma) or four (in epsilon and mu) constant domains (CH1 to CH4). IgA appears selectively in the seromucous secretions, where it clearly has the job of defending the exposed external surfaces of the body against attack by micro organisms. The IgA is synthesized locally by plasma cells and dimerized intracellularly together with a cysteine-rich polypeptide called J-chain.

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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.*