

Polyclonal Rabbit Anti-Human Chorionic Gonadotropin

Code A0231

Intended use

For In Vitro Diagnostic Use.

Polyclonal Rabbit Anti-Human Chorionic Gonadotropin, code A0231, is intended for use in immunohistochemistry. The antibody labels hCG-containing cells (1) and may be used for the demonstration of trophoblastic elements, e.g. in germ cell tumours (2). Differential identification is aided by the results from a panel of antibodies. Interpretation of results must be made within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and explanation

Human chorionic gonadotropin (hCG) is a 40 kDa glycoprotein composed of two subunits, α and β . The α subunit is common for hCG, luteinizing hormone (LH), follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH), while the β subunit is unique for hCG although its amino acid sequence partially is identical with that of the β subunit of LH (3, 4). hCG is the most important marker of gestational trophoblastic cells, being present in syncytiotrophoblastic cells and cells of the intermediate trophoblast but absent in cytotrophoblasts. hCG expression has also been demonstrated in some non-trophoblastic tumours, e.g. in 14% of patients with hepatocellular carcinoma (4). In pregnancy, the serum hCG concentration increases at an exponential rate initially, then peaks at around 100 kIU/L at about the tenth week after conception. The concentration decreases about tenfold over the next several weeks followed by a small increase near term (5).

Refer to Dako's *General Instructions for Immunohistochemical Staining* or the detection system instructions of IHC procedures for: 1) Principle of Procedure, 2) Materials Required, Not Supplied, 3) Storage, 4) Specimen Preparation, 5) Staining Procedure, 6) Quality Control, 7) Troubleshooting, 8) Interpretation of Staining, 9) General Limitations.

Reagent provided

Purified immunoglobulin fraction of rabbit antiserum provided in liquid form. In 0.1 mol/L NaCl, 15 mmol/L NaN₃.

Protein concentration g/L: See label on vial.

The titre variation between different lots of A0231 as determined by single radial immunodiffusion is less than 10%. This is achieved by adjusting the titre of each individual lot to match the titre of an antibody reference preparation kept at -80 °C.

Immunogen

The isolated beta-chain of hCG.

Specificity

The antibody reacts with hCG, and like most other polyclonal antibodies to beta-hCG it also shows a slight cross-reaction (about 4%) with LH (3). Traces of contaminating antibodies to human plasma proteins have been removed by solid-phase absorption.

In crossed immunoelectrophoresis only the hCG precipitation arch appears when using 12.5 μ L A0231 per cm² gel area against 10 μ L of concentrated urine from pregnant women (15th-16th week of gestation). No precipitation is seen when 12.5 μ L antibody per cm² gel area is tested against 10 μ L of normal human plasma. Staining: Coomassie Brilliant Blue.

As demonstrated by rocket immunoelectrophoresis, the antibody cross-reacts with the hCG-equivalent protein in hamster, horse, mouse, musk ox, polecat, swine, and wildebeest (6).

Precautions

1. For professional users.
2. This product contains sodium azide (NaN₃), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
3. As with any product derived from biological sources, proper handling procedures should be used.
4. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
5. Unused solution should be disposed of according to local, State and Federal regulations.

Storage

Store at 2-8 °C. Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the user must verify the conditions. There are no obvious signs to indicate instability of this product. Therefore relevant controls should be run simultaneously with patient specimens. If unexpected results are observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Dako Technical Support.

Specimen preparation

Paraffin sections: The antibody can be used for labelling paraffin-embedded tissue sections fixed in formalin. Pre-treatment of tissues with heat-induced epitope retrieval is required. Optimal results are obtained with Dako Target Retrieval Solution, code S1700, or 10 mmol/L Tris buffer, 1 mmol/L EDTA, pH 9.0. Less optimal results are obtained with Dako Target Retrieval Solution, High pH, code S3308, or 10 mmol/L citrate buffer, pH 6.0 and pre-treatment of tissues with proteinase K. The tissue sections should not dry out during the treatment or during the following immunohistochemical staining procedure.

Staining procedure

Dilution: Polyclonal Rabbit Anti-Human Chorionic Gonadotropin, code A0231, may be used at a dilution range of 1:300-1:600 when applied on formalin-fixed, paraffin-embedded sections of human placenta and using 20 minutes heat-induced epitope retrieval in Dako Target Retrieval Solution, code S1700, and 30 minutes incubation at room temperature with the primary antibody. Optimal conditions may vary depending on specimen and preparation method, and should be determined by each individual laboratory. The recommended negative control is Dako Rabbit Immunoglobulin Fraction (Solid-Phase Absorbed), code X0936, diluted to the same protein concentration as the primary antibody. Unless the

stability in the actual test system has been established, it is recommended to dilute the product immediately before use or dilute in Dako Antibody Diluent, code S0809.

Visualization: Dako LSAB™+/HRP kit, code K0679, and Dako EnVision™+/HRP kits, codes K4008 and K4010, are recommended. Follow the procedure enclosed with the selected visualization kit.

Automation: The antibody is well-suited for immunohistochemical staining using automated platforms, such as the Dako Autostainers.

Performance characteristics

Cells labelled by the antibody display a cytoplasmic staining pattern (1).









Normal tissues: In first trimester placenta, the antibody labels plentiful syncytiotrophoblast cytoplasmic granules, some undergoing exocytosis (1).

Abnormal tissues: 189 orchidectomy specimens with germ cell tumours (95 pure seminomas and 94 non-seminomas) were studied with the antibody. hCG was demonstrated in all choriocarcinoma components in the syncytiotrophoblasts and syncytiotrophoblast-like cells in 8% of seminomas and 30% of non-seminomas. Carcinoma-in-situ present in seminiferous tubules adjacent to the tumours was positive for hCG in a few cases (2).

References

1. Morrish DW, Marusyk H, Siy O. Demonstration of specific secretory granules for human chorionic gonadotropin in placenta. J Histochem Cytochem 1987;35:93-101.
2. Jacobsen GK, Jacobsen M. Alpha-fetoprotein (AFP) and human chorionic gonadotropin (HCG) in testicular germ cell tumours. A prospective immunohistochemical study. Acta Path Microbiol Immunol Scand Sect A 1983;91:165-76.
3. Mehta HC, MacDonald DJ. A sensitive enzyme immunoassay specific for human chorionic gonadotrophin. Clin Chim Acta 1982;121:245-50.
4. Leong AS-Y, Cooper K, Leong FJW-M. Manual of diagnostic antibodies for immunohistology; London: Oxford University Press; 1999. p. 25-6.
5. The National Committee for Clinical Laboratory Standards (NCCLS). Choriogonadotropin testing: Nomenclature, reference preparations, assay performance, and clinical application; Approved guideline. NCCLS document I/LA10-A (1-56238-314-0) NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA; 1996.
6. Hau J, Nilsson M, Skovgaard-Jensen H-J, de Souza A, Eriksen E, Wandall LT. Analysis of animal serum proteins using antisera against human analogous proteins. Scand J Lab Anim Sci 1990;17:3-7.

Explanation of symbols

 REF	Catalogue number	 2°C - 8°C	Temperature limitation		Use by
 IVD	In vitro diagnostic medical device	 LOT	Batch code		Manufacturer
	Consult instructions for use	 EC REP	Authorized representative in the European Community		



Agilent Technologies Singapore (International) Pte Ltd.
 No. 1 Yishun Avenue 7
 Singapore, 768923
 Tel. +44 161 492 7050
 www.agilent.com

Revision 2020.11