Novocastra™ Liquid Mouse Monoclonal Antibody CA125 (Ovarian Cancer Antigen)

Product Code: NCL-L-CA125

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Repetitive protein determinant expressed in the protein core of the CA125 human ovarian cancer antigen.
Clone	Ov185:1
Ig Class	IgG1
Antigen Used for Immunizations	A partially purified mucin fraction from a pool of cancer tissue from patients with epithelial ovarian cancer.
Hybridoma Partner	Mouse myeloma (P3x63-Ag8.653).
Preparation	Liquid purified Ig fraction presented in PBS (pH 7.6) with 1% BSA containing 15 mM sodium azide. Volume as indicated on vial label.
Effective on Frozen Tissue	Yes
Effective on Paraffin Wax Embedded Tissue	Yes (using the high temperature antigen unmasking technique: see overleaf).
Recommendations on Use	Immunohistochemistry: Typical working dilution 1:100. High temperature antigen unmasking technique. 60 minutes primary antibody incubation at 25 °C. Standard ABC technique. Western Blotting: No evaluated.
Positive Controls	Immunohistochemistry: Endometrium.
Staining Pattern	Extracellular membrane-associated.
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	CA125 is a type I membrane protein with a short intracellular domain, a transmembrane region and an extremely large, highly glycosylated extracellular domain. The extracellular domain is characterized by a region consisting of more than 60 tandem repeats of 156 amino acid motif flanked by an N-terminal sequence of 12,070 residues and a 229 amino acid membrane proximal region. The amino terminal sequence and the repeat domain are both rich in serine and threonine residues. Approximately one quarter of the molecular weight is estimated to be due to carbohydrate and both N-linked and O-linked glycan occur. Highly glycosylated O-linked repeats are typical of the mucin family of glycoproteins. Consequently, CA125 is also known as MUC16.
General References	O'Brien T J, Beard J B, Underwood L J, et al.. Tumour Biology. 23 (3): 154–169 (2002). O'Brien T J, Beard J B, Underwood L J, et al.. Tumour Biology. 22 (6): 345–347 (2001). Gabriel M, Obrebowska A and Spaczynski M. Ginekol Pol. 70 (11): 819–823 (1999). Alagoz T, Buller R E, Berman M, et al.. Gynecologic Oncology. 53: 93–97 (1994). Macri C I and Vasilev S A. Gynecologic and Obstetric Investigation. 37: 143–144 (1994). Ohmori T, Okada K, Tabei R, et al.. Pathology International. 4: 333–337 (1994). Rodriguez J M. Clinical Oncology. 6: 137 (1994). Ye C, Ito K, Komatsu Y, et al.. Gynecologic Oncology. 52: 267–271 (1994). Franchi M, Beretta P, Zanaboni F, et al.. Italian Journal of Gynaecology and Obstetrics. 5: 149–153 (1993).

Leica Biosystems Newcastle Ltd
Balliol Business Park West
Benton Lane
Newcastle Upon Tyne NE12 8EW
United Kingdom
✆ +44 191 215 4242

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.