Data Sheet
Topoisomerase I
mouse monoclonal antibody NCL-TOPO I

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	Human topoisomerase I.
Clone	1D6
Ig Class	IgG2b
Antigen used for immunisations	Prokaryotic recombinant protein corresponding to a region within the middle of the topoisomerase I molecule.
Hybridoma partner	Mouse myeloma (p3-NS1-Ag4-1).
Preparation	Lyophilised tissue culture supernatant containing 15mM sodium azide. Reconstitute with the volume of sterile distilled water indicated on the vial label.
Effective on frozen tissue	Yes. Zamboni's fixation recommended.
Effective on paraffin wax embedded tissue	Yes (using the high temperature antigen unmasking technique: see overleaf).
Recommendations on use	Immunohistochemistry: Typical working dilution 1:50 - 1:100. High temperature antigen unmasking technique. 60 minutes primary antibody incubation at 25^{o}C. Standard ABC technique. **Technical note:** The use of phosphate-containing wash buffers or diluents with this antibody has an adverse effect on staining. Only Tris-containing wash buffers or diluents should be used. Western Blotting: Not recommended.
Positive Controls	**Immunohistochemistry -** Tonsil.
Staining pattern	Nuclear
Storage and stability	Store unopened lyophilised antibody at 4^{o}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^{o}C. For long term storage, it is recommended that aliquots of the antibody are frozen at -20^{o}C (frost-free freezers are **not** recommended). Repeated freezing and thawing must be avoided. Prepare working dilutions on the day of use.

General Overview
Topoisomerases are nuclear enzymes involved in a variety of cellular activities such as chromosome condensation, DNA replication, transcription, recombination and segregation at mitosis. Human topoisomerase I is a 100kD protein capable of relaxing positively and negatively supercoiled DNA by performing a transient single-stranded nick which is then re-ligated at the end of the reaction. It has been shown that the enzyme is located in regions of the genome that are undergoing active RNA synthesis, where it probably reduces superhelical stresses in the DNA, enabling RNA polymerase to function properly. In normal eukaryotic cells, DNA topoisomerase I does not show relevant fluctuations across the cell cycle.

General References

Holden J A, Rahn M P, Jolles C J, et al.. Journal of Clinical Pathology-Molecular Pathology. **50**: 247-253 (1997).
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McLeod H L, Douglas F, Oates M, et al.. International Journal of Cancer. **59**: 607-611 (1994).
Negri C, Chiesa R, Cerino A, et al.. Experimental Cell Research. **200**: 452-459 (1992).
Shero J H, Bordwell B, Rothfield N F, et al.. Science. **231**: 737-740 (1986).

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. Deparaffinise 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 1500ml of the recommended unmasking solution (0.01M citrate buffer, pH6.0 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.
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 haematoxylin (if required), dehydrate and mount.

SOLUTIONS

1. **0.01M CITRATE BUFFER (pH 6.0)**

   Add 3.84 grams of citric acid (anhydrous) to 1.8 litres of distilled water. Adjust to pH 6.0 using concentrated NaOH. Make up to 2 litres with distilled water.

2. **1mM EDTA (pH 8.0)**

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

3. **20mM TRIS/0.65mM EDTA/0.005% TWEEN (pH9.0)**

   Dissolve 14.4g Tris (BDH product code 271197K) and 1.44g EDTA (SIGMA product code E-5134) to 0.55 litres of distilled water. Adjust pH to 9 with 1M HCl and add 0.3ml Tween 20 (SIGMA product code P-1379). Make up to 0.6 litres with distilled water. This is a 10x concentrate which should be diluted with distilled water as required (eg 150ml diluted with 1350ml of distilled water).

***** In most applications, 10mM 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.