Anti-Human Topoisomerase IIβ antibodies
(Polyclonal, cleared rabbit serum)

Product Description (Product Numbers HT2B11, HT2B15, HT2B21, HT2B25, HT2B31, and HT2B35)

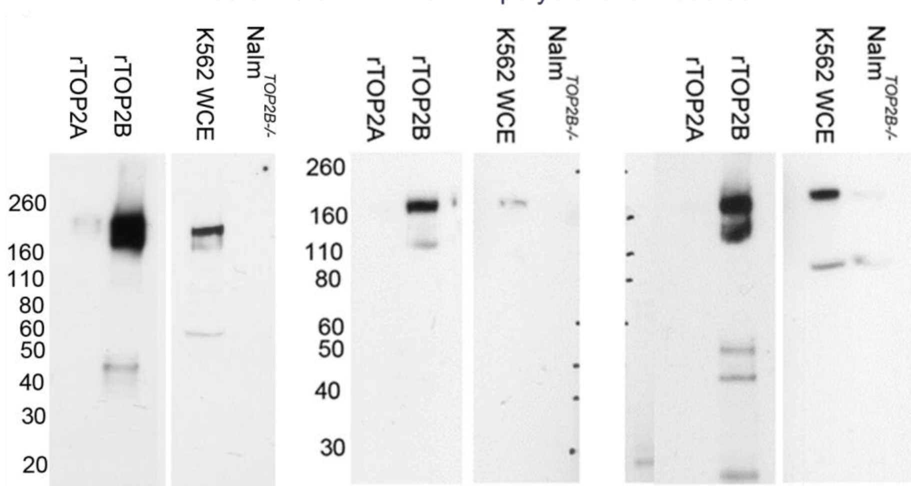
Topo IIβ polyclonal primary antibodies raised against GST-tagged TOP2B C-terminal domain (CTD) in cleared rabbit serum. For HTB2B11/15, the immunogen is the full-length CTD while HTB21/25 and HT2B31/35 were raised against a truncated (last third) CTD.
The antibodies are supplied at a concentration requiring ~1:1000 dilution for Western blotting and 1:400 dilution for Immunofluorescence[1,2] (e.g. for TARDIS assays[1]). The antibodies can be used for immunohistochemistry (IHC: ~1:800 dilution)[2].

For *in vitro* laboratory research use only.

Storage Buffer
Phosphate Buffered Saline (PBS) pH 7.2. Dilutions should be performed in PBS. Store at -20°C (stable for at least 12 months).

Western Blot
Whole cell extracts (WCEs) were prepared from K562 and $Nalm^{TOP2B-/-}$ cells. For gels, 10 µL of recombinant TOP2A and TOP2B were loaded along with 5 µL of the WCEs. Samples were run on NuSep 4-20% gels at 100 V and blotted onto hybond ECL nitrocellulose for 1 hour 100 V. After blocking (5% milk powder in TTBS) primary antibodies were added followed by anti-Rabbit HRP secondary antibody. The signal was developed using Biorad clarity ECL reagent and visualised by autoradiography.

Western blot: Anti-TOP2B polyclonal antibodies



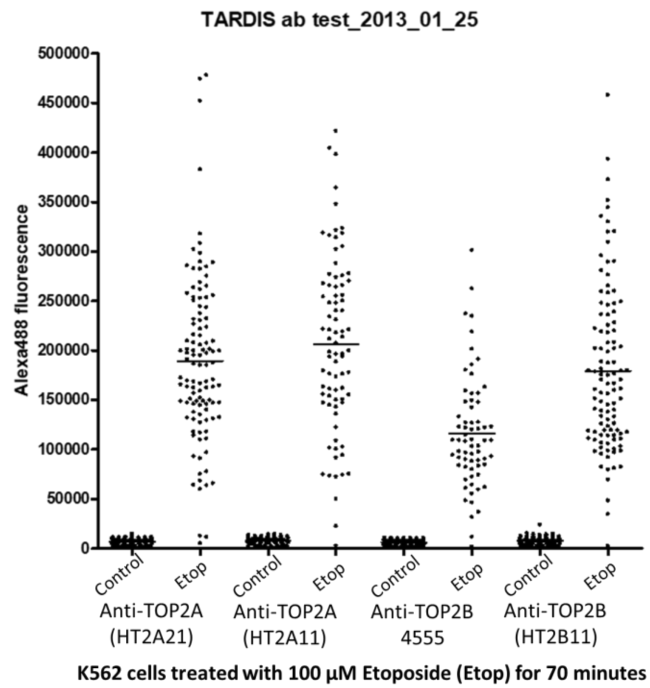
HT2B11/15
1:1000 dilution
Secondary:
1:5000 dilution
10s exposure

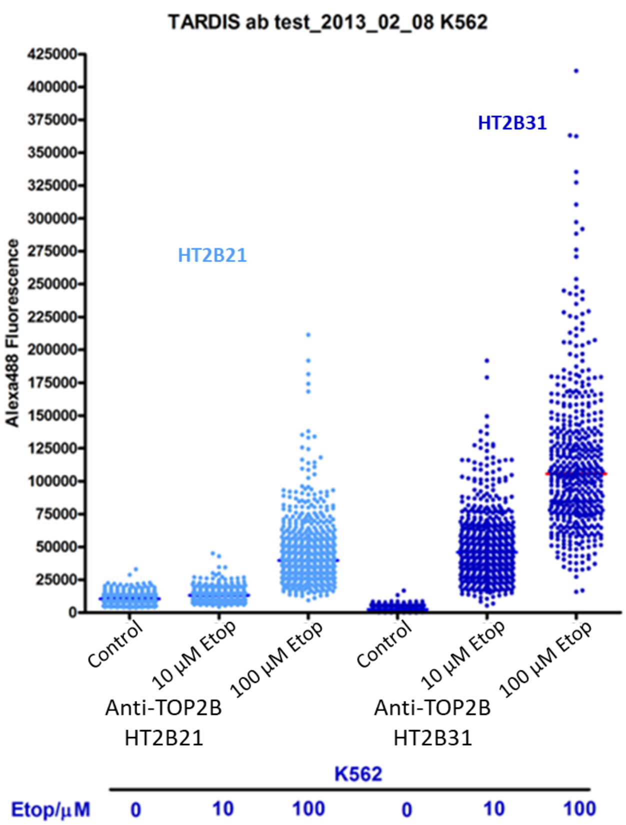
HT2B21/25
1:1000 dilution
Secondary:
1:5000 dilution
10s exposure

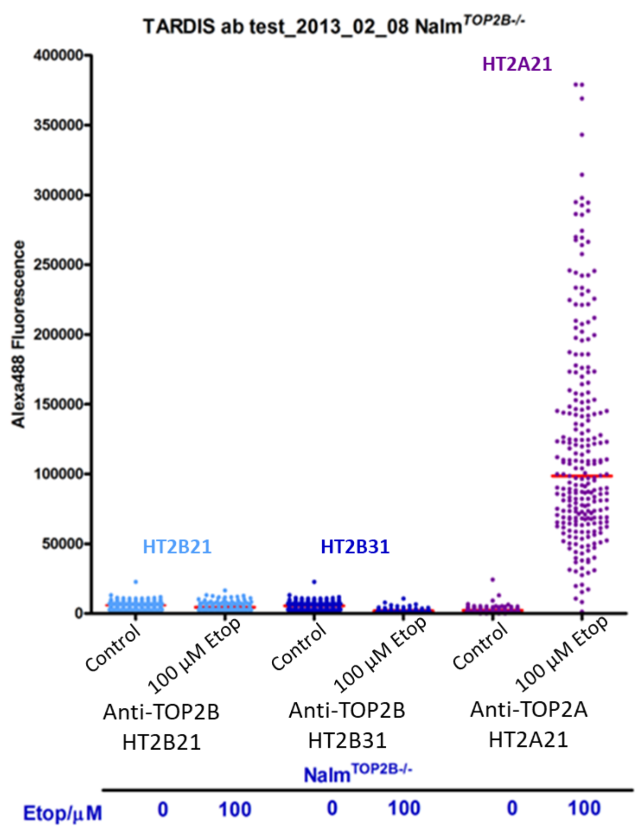
HT2B31/35
1:1000 dilution
Secondary:
1:5000 dilution
10s exposure

TARDIS Analysis

TARDIS analysis was carried out as per Reference 1, using either K562 or Nalm$^{TOP2B-/-}$ cells. The secondary antibody was an anti-Rabbit Alexa 488 G A-11034, diluted 1:250. Slides were mounted with Vectashield after Hoechst treatment. Cells were exposed to etoposide at the indicated doses for 60 min, unless otherwise indicated. All primary abs were used at 1:400.







References

1. Cowell IG, Austin CA. Visualization and Quantification of Topoisomerase-DNA Covalent Complexes Using the Trapped in Agarose Immunostaining (TARDIS) Assay. Methods Mol Biol. 2018;1703:301-316
2. Harkin, LF, *et al.* (2016), Distinct expression patterns for type II topoisomerases IIA and IIB in the early foetal human telencephalon. J. Anat., 228: 452-463.
3. Cowell IG, *et al*. Model for MLL translocations in therapy-related leukemia involving topoisomerase IIβ-mediated DNA strand breaks and gene proximity. Proc Natl Acad Sci U S A. 2012 Jun 5;109(23):8989-94.
4. Manville CM, *et al*. Genome-wide ChIP-seq analysis of human TOP2B occupancy in MCF7 breast cancer epithelial cells. Biol Open. 2015 Oct 12;4(11):1436-47