Human Topoisomerase (DNA) I (TOP1) ELISA Kit

Catalog No.: abx251860

Size: 96T

Range: 0.78 ng/ml - 50 ng/ml

Sensitivity: < 0.78 ng/ml

Storage: Store standard, detection reagent A, detection reagent B and the 96-well plate at -20°C and the rest of the kit components at 4°C.

Application: For quantitative detection of DNA in Human Serum, Plasma, Tissue Homogenates, Cell Culture Supernatants, Urine and other biological fluids.

Introduction: Human DNA topoisomerase 1 (TOP1) is a type 1B topoisomerase. TOP1 breaks a single strand of DNA, rotates it around the complimentary strand and decreases torsion, and then repairs the broken strand. DNA in cells forms supercoils that are more easily packaged but inhibit transcription; TOP1 unwinds these coils to allow polymerases to access the DNA more easily. TOP1 inhibitors are used in chemotherapy; they prevent TOP1 from repairing the broken strand, which can kill tumour cells when combined with DNA repair inhibitors.

Principle of the Assay
This kit is based on sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific to DNA is pre-coated onto a 96-well plate. The standards and samples are added to the wells and incubated. Biotin conjugated anti-DNA antibody is used as detection antibody. Next, Avidin conjugated to HRP is added. After TMB Substrate solution is added only wells that contain DNA, biotin-conjugated antibody and enzyme-conjugated Avidin will produce a blue colour product that changes into yellow after adding acidic stop solution. The intensity of the colour yellow is proportional to the DNA amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of DNA can be calculated.

Kit components
1. One pre-coated 96-well microplate (12 × 8 well strips)
2. Standard: 2 tubes
3. Sample Diluent: 20 ml
4. Wash Buffer (25X): 30 ml
5. Detection Reagent A (100X): 120 µl
6. Detection Reagent B (100X): 120 µl
7. Diluent Buffer A: 10 ml
8. Diluent Buffer B: 10 ml
9. Stop solution: 10 ml
10. TMB Substrate: 10 ml
11. Plate sealer: 5

Material Required But Not Provided
1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. Multi and single channel pipettes and sterile pipette tips
4. Squirt bottle or automated microplate washer
5. ELISA shaker
6. 1.5 ml tubes
7. Deionized or distilled water
8. Absorbent filter papers
9. 100 ml and 1 liter graduated cylinders
10. Deionised or distilled water

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Protocol

A. Preparation of sample and reagents

1. Sample

Isolate the test samples soon after collecting, analyze immediately or store at 4°C for up to 5 days. Otherwise, store at -20°C for up to one month or -80°C for up to two months to avoid loss of bioactivity. Avoid multiple freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid hemolysis and high cholesterol samples.
- **Tissue homogenates:** The preparation of tissue homogenates will vary depending upon tissue type – this is just an example. Rinse tissues with ice-cold PBS to remove the excess of blood. Weigh before homogenization. Finely mince tissues and homogenize with a tissue homogenizer on ice in PBS and sonicate the cell suspension. Centrifuge the homogenates at 5000 × g for 5 min and collect the supernatant. Assay immediately or aliquot and store at -20°C.
- **Cell culture supernatants:** Centrifuge at approximately 1000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.
- **Urine:** Aseptically collect the first urine of the day (mid-stream) voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
- **Other biological fluids:** Centrifuge at approximately 1000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

Note:

» Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used.

» Samples must be diluted so that the expected concentration falls within the kit's range.

» If the sample are not indicated in the manual's applications, a preliminary experiment to determine the validity of the kit will be necessary.

» Fresh samples or recently obtained samples are recommended to prevent protein degradation and denaturalization that may lead to erroneous results.

» Always use non-pyrogenic, endotoxin-free tubes for blood collection.

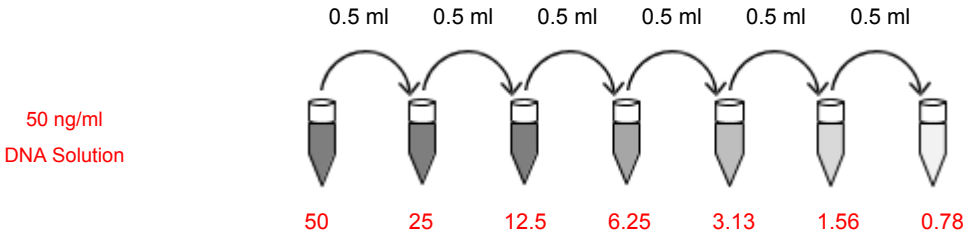
2. Wash buffer

Dilute the concentrated Wash buffer 25-fold (1/25) with distilled water (i.e. add 30 ml of concentrated wash buffer into 720 ml of distilled water).

3. Standard

Bring samples and all kit components to room temperature. Reconstitute the Lyophilized Standards with 1.0 ml of Sample Diluent buffer (kept for 10 min at room temperature) to make the 50 ng/ml Standard Solution. Allow the reconstituted standard to sit for 10 minutes with gentle agitation prior to carrying out the serial dilutions; avoiding foaming or bubbles. .**(Note: Do not dilute the standard directly in the plate).** Label 6 tubes with 25 ng/ml, 12.5 ng/ml, 6.25 ng/ml, 3.125 ng/ml, 1.5625 ng/ml and 0.78125 ng/ml respectively. Aliquot 0.5 ml of the Sample Diluent into each tube. Add 0.5 ml of 50 ng/ml standard solution into the 1st tube and mix thoroughly. Transfer 0.5 ml from 1st tube to 2nd tube, mix thoroughly, and so on.

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4. Detection Reagent A and B Preparation

Centrifuge Detection Reagent A and B briefly before use. Detection Reagent A and B should be diluted 100-fold with Diluent Buffer A and B respectively and mixed thoroughly. Pipette with a slow, smooth action to reduce volume errors. The solution should be prepared no more than 2 hours prior to the experiment. Please discard after use.

B. Assay Procedure

Equilibrate the kit components and samples to room temperature before use. It is recommended to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. It is recommended to measure each standard and sample in duplicate. Add the solution at the bottom of each well without touching the side walls.
2. Add 100 µl of the diluted standards into the standard wells. Aliquot 100 µl Sample Diluent to the control (zero) well.
3. Add 100 µl of appropriately diluted sample into the test sample wells. Add the solution at the bottom of each well without touching the side wall. Shake the plate mildly to mix thoroughly.
4. Seal the plate with a cover and incubate for 2 h at 37°C.
5. Remove the cover and discard the liquid. Do not wash.
6. Aliquot 100 µl of the detection Reagent A working solution to each well. Seal the plate with a cover and incubate for 1 h at 37°C.

   Note: If Detection Reagent A appears cloudy, warm the reagent to room temperature and mix gently until the solution appears uniform.
7. Remove the cover and discard the solution. Wash the plate 3 times with 1X Wash Buffer. Fill each well completely with Wash buffer (350µL) using a multi-channel Pipette or autowasher (1-2 minute soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.
8. Aliquot 100 µl of Detection Reagent B working solution into each well, seal and incubate at 37°C for 60 min.
9. Repeat the wash process 5 times as explained in step 7.
10. Aliquot 90 µl of TMB Substrate into each well. Seal the plate with a cover and incubate at 37°C for 15-30 min. Avoid exposure to light. The incubation time is for reference use only, the optimal time should be determined by end user. Do not exceed 30 min.
11. Add 50 µl of Stop solution into each well. There should be a colour change to yellow. Gently tap the plate to ensure thorough mixing.
12. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

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For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). Log-log curve fitting is recommended for data analysis. The DNA concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.

C. Precautions

1. Before using the kit, centrifuge the tubes briefly to bring down the contents trapped in the lid.
2. Wash buffer may crystallize and separate. If this happens warm to room temperature and mix gently until the crystals are completely dissolved.
3. Avoid foaming or bubbles when mixing or reconstituting components. Prepare the Standard solutions within 15 min of starting the experiment. Please use the diluted Standard for a single assay procedure and discard after use. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.
4. It is recommended measuring each standard and sample in duplicate.
5. Do not let the wells uncovered for extended periods between incubation. Once reagents are added to the wells, avoid letting the strips dry as this can inactivate the biological material on the plate. Incubation time and temperature must be controlled.
6. Ensure plates are properly sealed or covered during incubation steps.
7. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
8. Do not reuse pipette tips and tubes to avoid cross contamination.
9. Do not vortex the standard during reconstitution, as this will destabilize the protein. Once your standard has been reconstituted, it should be used right away. We do not recommend reusing the reconstituted standard.
10. The TMB Substrate solution is easily contaminated; work under sterile conditions when handling the TMB substrate solution. The TMB Substrate solution should also be protected from light. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

D. Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, medium and high levels of DNA were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of DNA were tested on 3 different plates, 8 replicates in each plate.

CV (%) = (Standard Deviation / mean) × 100

Intra-Assay: CV ≤ 4.5%

Inter-Assay: CV ≤ 7.4%

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