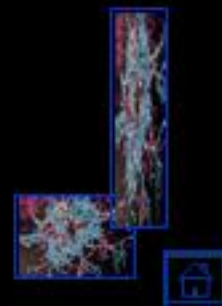


CHEMICON'S

Single-Stranded DNA
(ssDNA) Apoptosis
ELISA Kit (APT225)



ssDNA Apoptosis ELISA

Detects apoptosis with high sensitivity and specificity



Kit Components:

- Antibody Mixture: ssDNA Monoclonal & HRP-secondary
- Formamide
- Single-stranded DNA Positive Control
- Wash Buffer
- ABTS Solution (HRP substrate)
- Stop Solution



ssDNA Apoptosis ELISA

Materials required but not provided:

- 96-Well Microplate
- Nonfat dry milk
- Methanol/PBS
- S1 Nuclease

Equipment required:

- Adjustable volume pipettor with disposable tips
- 37°C Waterbath or Incubator



ssDNA Apoptosis ELISA

Advantages:

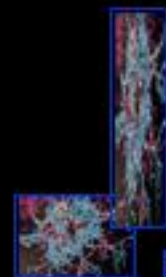
- HIGHLY SENSITIVE – Detect as few as 500 cells/well
- SPECIFIC FOR APOPTOSIS – Will not detect necrosis
- UNIVERSAL – Detects chromatin condensation, which is a hallmark of apoptosis
- VERIFIABLE – Positive control included



ssDNA Apoptosis ELISA

Assay Instructions

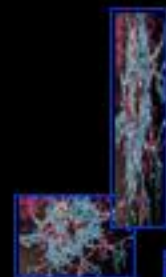
Step 1. Seed cells in microplate and induce apoptosis.



ssDNA Apoptosis ELISA

Assay Instructions

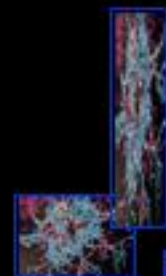
Step 2. Centrifuge plate at 200xg for 5 minutes.



ssDNA Apoptosis ELISA

Assay Instructions

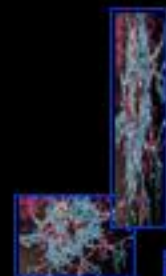
Step 3. Remove medium, add 200 μ L of fixative and incubate at room temperature for 30 minutes.



ssDNA Apoptosis ELISA

Assay Instructions

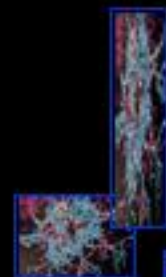
Step 4. Remove fixative and dry plates in 37°C waterbath for 20 minutes (or at room temperature for 1-2 hours).



ssDNA Apoptosis ELISA

Assay Instructions

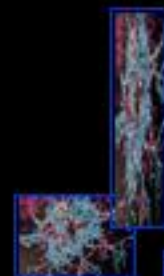
Step 11. Add 100 μ L of ABTS solution and incubate for 15-60 minutes to allow binding to HRP.



ssDNA Apoptosis ELISA

Assay Instructions

Step 6. Heat plates to 75°C in an oven without lid for 20 minutes (or in a circulating waterbath for 10 minutes) to denature DNA.



ssDNA Apoptosis ELISA

Assay Instructions

Step 7. Cool plates in refrigerator for 5 minutes, then remove formamide.



ssDNA Apoptosis ELISA

Assay Instructions

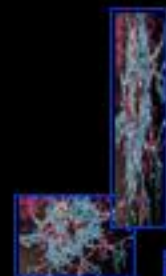
Step 8. Add 200 μ L of 3% nonfat dry milk in distilled water and incubate at 37°C for 1 hour. This will block non-specific binding sites.



ssDNA Apoptosis ELISA

Assay Instructions

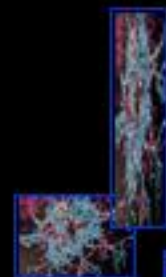
Step 9. Remove milk and add 100 μ L of Antibody Mixture. Incubate at room temperature for 30 minutes.



ssDNA Apoptosis ELISA

Assay Instructions

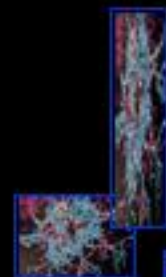
Step 10. Wash plates 3 times with wash buffer, using 250 μL per well per wash.



ssDNA Apoptosis ELISA

Assay Instructions

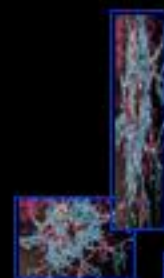
Step 5. Add 50 μ L of formamide and incubate at room temperature for 10 minutes.



ssDNA Apoptosis ELISA

Assay Instructions

Step 12. Add 100 μ L of Stop Solution and read absorbance in a standard microplate reader at 405 nm.



ssDNA Apoptosis ELISA

Assay Results

