5X Tris Borate EDTA (TBE) Buffer

CAT No. APL-1063

DESCRIPTION

APOLO's 5X Tris Borate EDTA (TBE) Buffer is widely used in DNA and RNA agarose gel electrophoresis and polyacrylamide gel electrophoresis. It has a higher buffering capacity than TAE buffer, and for this reason TBE is preferred over TAE during DNA synthesis. TBE buffer maintains the structural integrity of nucleic acids and more suitable for their size analysis. TBE buffer offers better resolution of 0.1 to 3 kb fragments; whereas, TAE (Tris Acetate EDTA) buffer provides better resolution of fragments greater than 4 kb. Furthermore, TBE is better suited for high-voltage (>150V) electrophoresis because of its higher buffering capacity and lower conductivity.

CONTENT

	APL-1063
5X Tris Borate EDTA (TBE) Buffer	1 L

COMPOSITION

5X Tris Borate EDTA (TBE) Buffer	Concentration
Tris Base	0.45 M
Boric Acid	0.45 M
EDTA.2Na	0.01 M

STORAGE

Store at RT.

FEATURES

APPLICATION

- Electrophoresis of nucleic acids in agarose and polyacrylamide gels
- Used both as a running buffer and a gel preparation buffer
- Filtered through a 0.22 μm membrane
- Recommended for electrophoresis of RNA and DNA fragments smaller than 1500 bp

PROTOCOL

Treating bacteria with PMA for qPCR

The following is a protocol for treating cultured laboratory strains of bacteria with PMA. Treatment of complex biological or environmental samples such as feces or soil may require optimization of sample dilution for PMA and light treatment.

1. Inoculate an appropriate media broth with bacteria (volume is dependent on size of experiment).
2. Shake cultures at 200 rpm at 37°C overnight.
3. Continuing culturing bacteria until the OD600 of the culture is approximately 1.
4. For dead cell control samples, heat inactivate bacteria at 58°C for 3 hours or 90°C for 5 mins. To confirm killing of bacteria, plate 10 µL of heat inactivated bacteria on the appropriate media plate, and 10 µL of a 1:100 dilution of control bacteria on another plate. Place the plates at 37°C and check for colony growth after 24~48 hours.
5. Pipette 500 µL aliquots of bacterial culture into clear microcentrifuge tubes.
6. Add the appropriate volume of PMA stock for a final concentration of 50 µM (e.g., 1.25 µL of 20 mM stock in 500 µL).
7. Incubate tubes in the dark for 5 minutes at room temperature. Flick tubes occasionally to mix, or incubate on a rocker covered with aluminum foil.
8. Expose samples to light to cross-link PMA to DNA. See Note 3 below for information on light sources.
9. Pellet cells by centrifuging at 5,000 x g for 10 minutes.
10. Extract genomic DNA for qPCR analysis using a standard protocol or commercially available kit. Use an appropriate protocol or kit for DNA extraction from complex biological or environmental samples (e.g., feces or soil).
11. Perform qPCR using primers against an appropriate genomic DNA target for your organism of interest. DNA templates modified with PMA will show delayed amplification by qPCR.

NOTES:

1. *Amplicons as short as 100 bp can be used, but longer target amplicons have been shown to decrease the signal from heat-killed PMA-treated cells.*
2. *Part of the proposed mechanism of action of PMA is the removal of PMA-bound DNA from samples via precipitation; therefore, the amount of input DNA in each sample should not be normalized between samples. Instead, PCR should be performed using equal volumes of gDNA eluate from each sample. For a positive control, 1 ng of live cell gDNA per reaction should be sufficient for achieving good signal. For gDNA extracted from bacterial cultures using a commercial extraction kit, 1~2 µL of eluted DNA can be used as a starting point for optimization.*
3. *For best results, we recommend that the photo-crosslinking be carried out on a PMA-Lite LED Photolysis Device. 15 minutes exposure should be sufficient for complete PMA activation. Commercial halogen lamps (>600 W) for home use have been employed for photo-activating PMA in some publications, though results have not been consistent due to inevitable variation in the set-up configurations. If you decide to use a halogen lamp, we recommend that you lay tubes on a block of ice set 20 cm from the light source, on a rocking platform to ensure continuous mixing. The ice block should be in a clear tray with a piece of aluminum foil under the clear tray*

to reflect the light upward. Set the lamp so that the light source is pointing directly downward onto the samples (up to 45° downward slant is acceptable). Expose samples to light for 5~15 minutes.

PRODUCT USE LIMITATION

Research use only.