Protocol

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NBT Assay: Brice Weinberg Protocol

Introduction

The nitroblue tetrazolium (NBT) assay is used to determine the ability of cells to produce reactive oxygen species, giving insight into their oxidative metabolism. During this assay, NBT is reduced and precipitates, resulting in dark blue granules (formazan). Phorbol myristate acetate (PMA) in this assay acts as a stimulant, inducing the reduction of NBT to form formazan. Here, we provide a general protocol for the investigation of oxidative metabolism in cells.

Materials

- Nitrotetrazolium blue chloride (NBT) (GoldBio Catalog # NBT)
- Phorbol myristate acetate (PMA)
- RPMI 1640
- PBS (GoldBio Catalog # P-271)
- Methanol
- Molecular biology grade water
- Safranin O

Preparation of NBT Solution

- 3 mg of NBT
- 10-20 µg/ml of PMA
- 1.5 ml of RPMI 1640

Storage and Handling

- Store NBT at 4°C and protect from light.
- This product should be stored immediately upon arrival at 4°C.

Method

1. Take a sample of about 1.0 x 10^6 cells total and wash once with PBS in a 5 ml tube.

2. Suspend the pellet in 1 ml of NBT solution and incubate at 37°C for 15 minutes.

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3. Wash with 1 ml of cold PBS and spin at 1500 rpm for 5 minutes, repeat this wash 3 times.

4. Resuspend the final pellet in 200 µl PBS and load 100 µl into a slide well.

5. Spin the slide at 3000 rpm for about 1 minute and allow the slide to dry.

6. Fix slide with methanol for 1 minute, rinse with molecular biology grade water, and counterstain with 0.15% Safranin O (prepared with distilled water) for 30 seconds to 1 minute.

7. Finally, rinse off excess stain and allow slide to dry.

Associated Products

- Nitrotetrazolium blue chloride (NBT) (GoldBio Catalog # NBT)
- PBS (GoldBio Catalog # P-271)

References

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