

Tribo™ Alkaline Phosphatase (AP) Blue Staining Kit (Catalog# TBS2085)

DESCRIPTION

Alkaline phosphatase (AP) is a membrane bound enzyme synthesized by cells in many tissues, especially bone, liver and stem cells. It hydrolyzes phosphate containing molecules under alkaline conditions. AP is widely used as a universal pluripotent marker to identify undifferentiated state for all types of pluripotent stem cells including embryonic stem cells, embryonic germ cells, and induced pluripotent stem cells. The undifferentiated state of stem cells can be characterized by high level of AP expression, along with the expression of multiple pluripotency markers including the transcription factors Nanog, Oct4, Sox2, SSEA-1, SSEA-3/4, and tumor related antigens, TRA-1-60, TRA-1-81.

Tribo™ alkaline Phosphatase Staining Kit is a specific and sensitive tool for the phenotypic assessment of stem cell differentiation by the determination of AP expression.

Directions for Use

Detect undifferentiation of stem cell as a stem cell marker.

KIT COMPONENT

AP Blue staining Part A: 15 mL

AP Blue staining Part B: 15 mL

Storage

Store all components at 4°C for 12 months.

Materials Required, But Not Supplied

Fix Solution: 4% paraformaldehyde Solution.

1x PBS

1x PBST

Microscope

PREPARATION OF REAGENTS

Prepare Fresh 1x AP staining solution by mixing equal volume of AP Staining Solution A and Solution B (1:1). The volume of AP staining solution needed is based on the number of samples. For example, each well needs 0.4 mL for 24-well plate.

STAINING PROTOCOL

1. Aspirate the culture medium and wash the cells twice with 1 mL of PBST.
2. Add 0.5 mL of Fix Solution to each well for a 24-well plate. Incubate at room temperature for 2 min.
3. Remove the Fix Solution and wash the fixed cells twice with 1 mL PBST.
4. Aspirate the final wash, then, add 0.4 mL per well of freshly prepared AP staining solution.
5. Incubate the cells at room temperature for 15-30 min, protected from light.
6. Remove the AP Staining Solution, and then wash the stained cells twice with 1 mL of 1x PBS
7. Cover the cells with 1 x PBS or mounting medium to prevent drying.
8. Count the blue stained cell colonies (undifferentiated stem cells) vs. colorless colonies (differentiated cells) using a light microscope.

REFERENCES

Draper J. et al. J Anat. 200: 249-258 (2002)

Yu J., et al. Science 318: 1917-1920 (2007)

RELATED PRODUCTS

AP Red Staining Kit (catalog# TBS2080)

Resazurin Cell Viability Kit (TBS2001)

ATP Colorimetric/Fluorometric Assay (TBS2010)

ADP Colorimetric/Fluorometric Assay Kit (TBS2020)

CCK-8 Cell Viability Assay (TBS2022)

Thiol Fluorometric Assay (TBS2026)

GSH Assay (TBS2028)

Caspase-3 Colorimetric Assay kit (TBS2030)

AHCY Activity Fluorometric Assay (TBS2056)

Glucose Oxidase Colorimetric/Fluorometric Assay (TBS2088)

Homocysteine Fluorometric Assay (TBS2091)

NNMT Inhibitor Screening Assay (TBS2097)

NNMT Activity Fluorometric Assay (TBS2098)

G6PDH Activity Colorimetric Assay (TBS2102)

AHCY Inhibitor Screening Fluorometric Assay (TBS2099)

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