Tribo™ Alkaline Phosphatase Staining Kit I (Red) (Catalog# TBS2080)

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 an 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 such as the transcription factors Nanog, Oct4, Sox2, SSEA-1, SSEA-3/4, and tumor related antigens, TRA-1-60 and 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 COMPONENTS:

AP Staining Solution A Red: 15 mL
AP Staining Solution B Red: 15 mL
Fix Solution: 25 mL

Storage: Store all components at 4°C for 6 months.

Materials Required But Not Supplied

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 and 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 1x PBS or mounting medium to prevent drying.
8. Count the red 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 Staining Kit II (Blue; catalog# TBS2085)
PBST-10x (catalog# TBS5011)
FBS(catalog# TBS 8001)
Gelatin Solution (catalog# TBS8004)
Protein Loading Buffer (catalog# TBS5014)

This product is for *in vitro* research use only and is not intended for use in humans or animals in therapeutic or diagnostic procedures.

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