Tartrate Resistant Acid Phosphatase (TRAP) staining Kit

Introduction

The acid phosphatase (ACP) family is a class of enzymes that are widely found in various tissues, mainly in the lysosomes of cells, so they are often used as markers of lysosomes. Tartrate-resistant acid phosphatase (TRAP) is the most dominant enzyme in the ACP family, found in alveolar macrophages in normal humans and splenocytes in patients with leukemia, and is usually located in the follicles of cells and is not released into the blood. TRAP in the blood is mainly derived from osteoclasts, therefore, by measuring the level of TRAP in the blood, the activity of osteoclasts can be assessed.

The Tartrate Resistant Acid Phosphatase (TRAP) staining kit uses naphthol AS-BI as a substrate, which can be catalyzed by TRAP in an acidic environment to produce phosphoric acid and naphthol. Subsequently, naphthol reacts with diazonium salts to form colored conjugates and is detected. This kit can be used for fresh blood smears, cell smears, and can also be used for the detection of frozen sections and paraffin sections.

Components and Storage

Components	K2606-10 mL	K2606-20 mL
GBC Solution A	100 μL	200 μL
GBC Solution B	100 μL	200 μL
AS-BI Staining Solution	100 μL	200 μL
TRAP Buffer	9.7 mL	19.4 mL
Hematoxylin Staining Solution	10 mL	20 mL
Methyl Green Staining Solution	10 mL	20 mL

Store TRAP Buffer and Methyl Green Staining Solution at room temperature away from light. Store Hematoxylin Staining Solution at 4°C away from light. Store other reagents at -20°C away from light. This kit is stable for 3 months.

Protocol

1. **Preparation before staining:** Mix 100 μL of GBC Solution A and 100 μL of GBC Solution B with gentle stirring for 30 s to make the GBC Solution, and then let stand for 2 minutes.

2. **Sample Preparation:**

   1) Cell smear: Take fresh whole blood or bone marrow and prepare smears according to routine operations

for later use.

2) Adherent cells/cells in coverslip: Discard the medium and wash 3-4 times with PBS. Then drain the excess water.

3) Frozen sections: Warm the sections to room temperature, immerse them in distilled water and wash for 1-2 min, then drain the excess water.

4) Paraffin sections: Dewax 2 times for 5-10 min each time. Absolute ethanol for 5 min, 90% ethanol for 2 min, and 70% ethanol for 2 min. Immerse in distilled water and wash for 2 min. Drain excess water.

3. **Staining:**

1) Fix with a pre-chilled fixative solution for 30 s-3 min, usually 30-60 s is enough.

***Note:** This kit does not provide a fixative solution. Common 4% paraformaldehyde can be used or self-prepared (25 mL citrate solution, 65 mL acetone, 8 mL 37% formaldehyde, 2 mL H_2O).

2) Wash with distilled water, then remove excess water (not too dry).

3) Mix AS-BI Staining Solution: GBC Solution: TRAP Buffer in a 1: 2: 97 ratio to make TRAP staining solution. Then heat the TRAP staining solution to 37°C. prepare fresh TRAP staining solution every time.

4) For slices: Drip pre-warmed TRAP incubation solution onto the slices and place them in a wet box in a 37°C incubator for 45-60 min in the dark. Wash 3 times with distilled water.

For adherent cells: Drip pre-warmed TRAP incubation solution to cover the cells and place cells in a 37°C incubator protected from light for 45-60 min. Wash 3 times with distilled water.

5) Counterstaining: Methyl Green Staining Solution for 2-3 min. Or Hematoxylin Staining Solution for 5 min, then tap water for 10 min to return to blue.

***Note:** If using Methyl Green Staining Solution for counterstaining, there is no need to return to blue.

3. **Detection:**

1) Sections: Wash with distilled water, drain the excess water, add a water-based mounting and observe under the microscope.

2) Adherent cells: Wash with distilled water, and observe directly under the microscope with water or PBS.

4. **Analyze：**

Positive particles	Purplish-red
Nucleus	Blue (Hematoxylin) or Green (Methyl Green)

Note

1. The reaction substrates and dyes in this kit are easy to be invalid, and it is recommended to store the product

according to the recommended storage conditions in the manual as soon as possible when receipt.

2. Enzyme activity is easily affected by various factors and decays or is small, so it is recommended to take fresh samples for preparation. At the same time, when the fixative solution is fixed at 4°C, the fixation time should not exceed 24 h. It is recommended to use a low melting point wax (52-56°C) for embedding to avoid thermal inactivation.

3. For your safety and health, please wear lab coats and gloves during the experiment.

4. For research use only. Not to be used in clinical diagnostic or clinical trials.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com