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抗酒石酸酸性磷酸酶染液（TRAP）

货号：R27216

产品组成：4×10ml，4×20ml

名称		4×10ml	4×20ml	储存条件
试剂 A TRAP 固定液		50ml	100ml	2-8° C,避光
试剂(B): TRAP 缓冲液	B1:AS-BI 染色液	1ml	2×1ml	-20° C,避光
	B2: GBC 染色液	0.1ml	0.2ml	-20° C,避光
	B3: TRAP 缓冲液	9ml	18ml	室温，避光
临用前，按 B1:B2:B3=10:1:90混合，即为 TRAP 孵育液，即配即用。				
试剂(C):苏木素染色液		10ml	20ml	2-8° C,避光
试剂(D);:甲基绿染色液		10ml	20ml	室温,避光

产品介绍:

酸性磷酸酶(acid phosphatase, ACP)分布极广泛，遍布各种组织，主要存在于细胞的溶酶体内，所以常作为溶酶体标志酶。溶酶体外的酸性磷酸酶存在于内质网和胞质内。各种动物中的酸性磷酸酶各有不同，酸性磷酸酶的适宜 pH 为4.5-5.5。存在于正常人肺泡巨噬细胞和白血病人脾脏的抗酒石酸酸性磷酸酶(Tartrate-resistnt acid phosphatase, TRAP)均在细胞滤泡中，并不是释放入血液。血液中的 TRAP 绝大多数来源于破骨细胞，因此可以通过测量血液中的 TRAP 了解破骨细胞的功能状态。抗酒石酸酸性磷酸酶染色试剂盒以萘酚 AS-BI 为底物，在酸性 pH 下被酸性磷酸酶水解释放出磷酸和茶酚，茶酚与重氮盐偶联生成有色产物，定位于细胞质中，若细胞内的 ACP 有抗酒石酸的活性，则呈阳性反应。该染色液可用于新鲜血涂片、细胞涂片，亦可用于冰冻切片、石蜡切片。

自备材料: 蒸馏水、恒温箱、载玻片、推玻片、光学显微镜

操作步骤:（仅供参考）

（一） 血液、 细胞涂片:

1、 推片:取新鲜血液或骨髓涂片置于载坡片上，推玻片于载玻片保持30度，置于血液或细胞滴液的正前方，稍往后移不血液或细胞滴液接触使后者沿

推片下缘散开，再匀速沿载玻片平面平稳向前滑动至铺满血膜为止。

2、 自 然晾干，TRAP 固定液2-89C 固定30s-3min，多数情况下30-60s 即可。

3、 水洗， 稍微晾干(不宜过分干燥)。

4、 切片入 TRAP 孵育液，置于37° C 温箱， 避光浸染45-60min，水洗。

5、 复染：苏木素染色液染色5min 或甲基绿染色液染色2-3min。

6、 水洗、晾干、镜检。

(二)冰冻切片：

1、 冰冻切片回温至37° C， 水中浸泡1-2min。

2、自 然晾干，TRAP 固定液2-89C 固定1-3min。

3、水洗，稍微晾干(不宜过分干燥)。

4、切片入 TRAP 孵育液，置于379C 温箱，避光浸染45-60min，水洗。

5、复染：苏木素染色液染色5-8min 或甲基绿染色液染色2-3min。

6、水洗、晾干、镜检。

(三)石蜡切片：

1、石蜡切片脱蜡5-10min， 重复一次。

2、无水乙醇5min，90%乙醇和70%乙醇各2min，蒸馏水浸洗2min。

3、自 然晾干，TRAP 固定液2-89C 固定30s-3min，多数情况下30-60s 即可。

4、水洗，稍微晾干(不宜过分干燥)。

5、切片入 TRAP 孵育液，置于379C 温箱，浸染45-60min， 水洗。

6、复染:苏木素染色液染色5-8min 或甲基绿染色液染色2-3min。

7、水洗、晾干、镜检。

阳性颗粒	紫红色
细胞核	蓝色(苏木素)或绿色(甲基绿)

临床意义：

1、 毛细胞白血病的毛细胞 ACP 染色呈强阳性或中度阳性，且不被酒石酸抑制，其他细胞均呈阴性或极弱阳性。

2、 急性白血病幼单核细胞 ACP 染色呈阳性，原淋巴细胞星弱阳性，原粒细胞对 ACP 反应不一”。

3、 T 淋巴细胞 ACP 染色呈阳性，颗粒粗大、分布密集。B 淋巴细胞呈阴性或颗粒细小的弱阳性。

4、 戈谢细胞 呈强阳性，尼曼-皮克细胞呈阴性或弱阳性。

注意事项:

1、 TRAP 孵育液易失效，本法宜用皮肤穿刺血涂片，晾干后应及时染色。

2、 对冰冻切片染色时，应减少切片在室温暴露的时间。

3、 样本需新鲜，取材后应立即处理，否则会影响酶的活性。

4、 组织固定 需在2-8″C 冰箱进行，时间不宜超过24h，否则酶活性会减弱或消失。

5、 组织在石蜡包埋时， 温度不宜高于56° C.应使用熔点为52-52-8″C 的石蜡进行浸蜡，浸蜡时间要短，否则酶活性会减弱或消失。

6、 不纯的二甲苯会分解黑色沉淀，宜选用 AR 级以上的二甲苯。

7、 为了您的安全和健康，请穿实验服并戴一次性手套操作。

保质期：3个月

源叶生物

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Tartrate-Resistant Acid Phosphatase (TRAP) Stain Kit

Size: 4×10mL/4×20mL
Storage: -20℃,avoid light,valid for 3 months.

Kit Components

Reagent		4×10mL	4×20mL	Storage
Reagent(A): TRAP Fixative		50mL	100mL	2-8℃, avoid light
Reagent(B): TRAP Incubation Solution	B1: AS-BI Buffer	1mL	2×1mL	-20℃, avoid light
	B2: GBC Solution	0.1mL	0.2mL	-20℃, avoid light
	B3: TRAP Buffer	9mL	18mL	RT, avoid light
Mix reagent B1,B2,B3 in 10:1:90 ratio as TRAP Incubation Solution before use and not store for long.				
Reagent(C): Hematoxylin Solution		10mL	20mL	2-8℃, avoid light
Reagent(D): Methyl Green Solution		10mL	20mL	RT, avoid light

Introduction

Acid phosphatase (ACP) is widely distributed in various tissues, mainly in the lysosome, so it is often used as a lysosome marker enzyme. The acid phosphatase outside of lysozyme exists in endoplasmic reticulum and cytoplasm. Acidic phosphatase is different in all kinds of animals. The optimum pH for its activity is 4.5-5.5. Tartrate-resistant acid phosphatase (TRAP), which mainly exists in normal human alveolar macrophages and leukemia human spleen cells, is released into the cell follicles rather than into the blood. The majority of TRAP in blood comes from osteoclasts, so we can know the function of osteoclasts by measuring TRAP in blood.

The reaction principle of Tartrate-Resistant Acid Phosphatase (TRAP) Stain Kit is that phosphoric acid and naphthol are released by hydrolysis of acid phosphatase with AS-BI as substrate at acidic pH. Naphthol was coupled with diazo salts to form colored products, which were localized in the cytoplasm. If intracellular ACP has tartaric acid-resistant activity, it is positive. It usually used for fresh blood smear, cell smear, frozen section, etc.

Self Provided Materials

Distilled Water, Incubator, Slide, Microscope

Protocol(*for reference only*)

For Blood or Cell Smear

1. Dry blood or cell smear in air then fix in TRAP Fixative at 4 ℃ for 30s-3mins and usually for 30-60s.
2. Wash with distilled water and slightly dry in air.
3. Add the TRAP Incubation Solution to sections and place in 37℃ incubator and incubate in dark for 45-60min.Then wash with distilled water.
4. Re-dyeing with Hematoxylin Solution or Methyl Green Solution for 2-3min.
5. View the sections under microscope after washing or sealing.

For Frozen Section

1. Restore the section to 37℃ by immerse in water for 1-2min.
2. Dry in air and fix in TRAP Fixative for 1-3mins at 2-8℃.
3. Wash with distilled water and slightly dry in air.
4. Add the TRAP Incubation Solution to sections and place in 37℃ incubator and incubate in dark for 45-60min.Then wash with distilled water.
5. Re-dyeing with Hematoxylin Solution or Methyl Green Solution for 2-3min.
6. View the sections under microscope after washing or sealing.

For Paraffin Section

1. Dewax paraffin sections by xyline twice for 5-10min each.Then rehydrate in graded Echanol（include 5min in 100%Echanol ， 2min each in 90%Echanol and 70%Echanol）.
2. Finally wash with distilled water for 2min，slightly dry in air.
3. Add the TRAP Incubation Solution to sections and place in 37℃ incubator and incubate in dark for 45-60min.Then wash with distilled water.
4. Re-dyeing with Hematoxylin Solution or Methyl Green Solution for 2-3min.

5. View the sections under microscope after washing or sealing.

Result

Positive	Purplish Red
Nucleus	Blue or Green

Clinical Significance

1. TRAP staining of hair cells in hairy cell leukemia is strongly or moderately positive, and is not inhibited by tartaric acid. The other cells are negative or especially weak positive.
2. TRAP staining is positive in immature monocytes of acute leukemia, and weak positive in prolymphocytes. The response of progranulocytes to TRAP is different.
3. T lymphocyte TRAP staining is positive, with large and densely distributed granules. B lymphocyte is negative or weak positive with small granules.
4. Gaucher cells are strongly positive and Niemann-Pick cells are negative or weak positive.

Note

1. TRAP Incubation Solution is easy to lose effect. It's recommended to use skin puncture blood smears and stain quickly after drying.
2. When staining frozen sections, the exposure time of sections at room temperature should be reduced.
3. Samples should be fresh and treated immediately after sampling, otherwise the enzyme activity will be affected.
4. Tissue should be fixed in refrigerator at 2-8℃ no more than 24 h, otherwise the activity of enzyme will be weakened or disappeared.
5. When embedding the tissue in paraffin, the temperature should not be higher than 56℃. It's recommended to use paraffin wax with melting point of 52-52-8 ℃ for wax soaking. The soaking time should be short, otherwise the enzyme activity will weaken or disappear.
6. The impure dimethylbenzene will decompose the black precipitation, and the xylene above AR grade should be selected.
7. For your safety and health, please wear experimental clothes and disposable gloves.