VitroView™ Myofibrillar ATPase Stain Kit
(For 50~100 slides)
SKU# VB-3030

Introduction

The calcium method for ATPase demonstration, utilizing solutions with varying pH levels, has primarily served to differentiate muscle fiber types. These fibers are commonly classified broadly as type 1 ("slow, red muscle, oxidative") and type 2 ("fast, white muscle, glycolytic"). Type 2 muscle fibers can be further categorized as 2a (glycolytic), 2b (glycolytic/oxidative), and 2c, which are believed to represent fibers transitioning between types due to disease or injury.

The staining process is believed to function as follows: the pre-incubation pH deactivates the myosin-ATPase enzyme specific to certain fiber types. The remaining active enzyme binds to a calcium atom, which is subsequently replaced by cobalt and ultimately precipitated as a black insoluble compound by ammonium sulfide. VitroView™ Myofibrillar ATPase Stain Kit is designed to distinguish muscle fiber types.

Kit Components

SKU#	Reagent	Size
VB-3030-1	4.3 Pre-Incubation Solution	1.5 ml ×5
VB-3030-2	4.6 Pre-Incubation Solution	1.5 ml ×5
VB-3030-3	9.4 Pre-Incubation Solution	1.5 ml ×5
VB-3030-4	ATP Incubation Solution	1.5 ml ×5
VB-3030-5	1% Calcium Chloride	250 ml
VB-3030-6	2% Cobalt Chloride	1.5 ml ×5
VB-3030-7	0.1 M Sodium Barbital	250 ml
VB-3030-8	2% Ammonium Sulfide	1.5 ml ×5

Storage

Store at -20°C

Procedures

Tissue preparation for cryosectioning

1. Sacrifice the animal by either cervical dislocation or decapitation, in accordance with available ethical permit.
2. Quickly collect tissues of interest without fixation, and rapidly freeze on dry ice (tissues may require freezing in isopentane) or propane chilled with liquid nitrogen to obtain optimal morphology).
3. Store tissues in aluminum foil at -80 °C until ready to section.
4. Embed frozen tissue in preparation for cryosectioning.
5. Collect 10-16µm cryostat sections. Thaw sections onto slides at room temperature for 2-5 minutes, and store slides without cover-slipping at -20 °C until ready to use.

Staining Procedure

1. For each samples, three frozen sectioned slides are needed.

2. Apply 80-200µl of the 4.6 Pre-Incubation Solutions, 4.3 Pre-Incubation Solution, and 9.4 Pre-Incubation Solution onto each slide to completely cover the sections. Incubate for exactly 5 minutes for the 4.3 and 4.6 Pre-Incubation Solutions and for 15 minutes for the 9.4 Pre-Incubation Solution at room temperature.
3. After the appropriate pre-incubation time, discard the solution and rinse once with deionized water.
4. Add 80-200µl of ATP Incubation Solution onto the slide and incubate for 25 minutes for the 4.3 Pre-Incubation Solution slides and 4.6 Pre-Incubation Solution slides, and 15 minutes for the 9.4 Pre-Incubation Solution slides.
5. Wash each staining slide with 3 changes of 1% Calcium Chloride for a total duration of approximately 10 minutes.
6. Add 2% Cobalt Chloride to each slide for 10 minutes.
7. Wash with 3-5 changes using a 5mM Sodium Barbital solution. Note: the initial wash should display a faint blue color.
8. Rinse with 5 changes of deionized H2O.
9. The Next STEP SHOULD BE EXECUTED IN A FUME HOOD due to the presence of noxious and toxic fumes:
    A. Add 2% ammonium sulfide solution to each slide for 20 - 30 seconds (sections will appear very dark).
    B. Rinse in the fume hood with approximately 5 changes of tap water.
10. Dehydrate with 2 changes of 95% Ethanol and 2 changes of 100% Ethanol (2 minute per change).
11. Clear with 3 changes of xylene (5 minutes per change) and coverslip with CANADA BALSAM.

Positive Control: Snap frozen striated muscle

Expected Results

Pre-Incubation Solution, pH	TYPE 1	TYPE 2A	TYPE 2B	TYPE 2C
9.4	light (0 +1)	dark (+3)	dark (+3)	dark (+2)
4.6	dark (+3)	light (0)	intermediate (+1 +2)	intermediate (+1 +2)
4.3	dark (+3)	light (0)	light (0)	intermediate (+1 +2)

References

1. Brooke MH, Kaiser, KK. Arch. Neurol., 23: 369 - 379, Oct. 1970.
2. Brooke MH, Kaiser KK. J. Histochem. Cytochem., 18: 670 - 672, 1970.
3. Dubowitz V, Brooke MH. MUSCLE BIOPSY: A MODERN APPROACH, W.B. Saunders Co., Ltd, London, 1973.
4. Sheehan and Hrapchak, HISTOTECHNOLOGY, 2nd Edition; Batelle Press, Columbus, 1987.
5. Planer GJ, Pestronk A, et. al., Muscle & Nerve 1992; 15:258.

Note: This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Precautions: Handle with care in chemical hood. Avoid contact with eyes, skin and clothing. Do not ingest. Wear gloves.