TTC Staining Kit, 4%

Catalog No.: RA20113

Basic Information

Product name	TTC Staining Kit, 4%
Sizes	100 mL
Storage	2-8 ℃, keep away from light
Shipping	Shipped with ice pack
Validity	6 months

Product Introduction

2,3,5-Triphenyltetrazolium chloride (TTC) has a molecular weight of 334.80, molecular formula $C_{19}H_{15}ClN_4$, and CAS number 298-96-4. TTC is a lipophilic, light-sensitive compound originally used to assess seed viability and later applied to visualize ischemic infarction in mammalian tissues. TTC acts as a proton acceptor for the pyridine-nucleotide-linked enzyme system of the respiratory chain. In normal tissue it is reduced by respiratory enzymes to a red product, whereas ischemic tissue, in which respiratory enzyme activity is decreased, remains pale and unchanged. Thus TTC staining provides a macroscopic method for evaluating dehydrogenase activity in tissues.

EnkiLife TTC Staining Solution (4%) is routinely used for fresh cardiac and brain tissues obtained at autopsy and for early infarct visualization in experimental animal models; it can also be employed to determine seed and pollen viability. TTC produces a red formazan upon reaction with dehydrogenases in viable tissue, allowing assessment of tissue or seed viability based on staining location and intensity.

Materials Required (Not Supplied)

1. Normal saline, 4% paraformaldehyde or 10% neutral buffered formalin.
2. Glass slides, coverslips, incubator, microscope, low-temperature freezer.

Perimental procedure

(I) Brain Tissue Staining

1. Harvest fresh brain tissue (under anesthesia or after saline perfusion) and snap-freeze at –20 °C for 20–30 min to facilitate sectioning.
2. Section the tissue: 2–3 mm thickness for animal samples, 3–5 mm for human samples; collect 4–5

consecutive slices. First cut: midpoint between anterior pole and optic chiasm. Second cut: optic chiasm. Third cut: infundibular stalk. Fourth cut: between infundibular stalk and posterior pole (see Zhang Juntian, ed., Modern Pharmacological Experimental Methods).

3. Immerse slices in TTC Staining Solution (4%) and incubate 30–35 min protected from light.
4. Fix slices in 4% paraformaldehyde or 10 % neutral buffered formalin for 4–24 h.
5. Blot excess liquid and quantify infarct volume with image-analysis software (e.g., IPP).

(II) Seed Staining

1. Soak seeds in warm water (30–35 °C) for 2–6 h to allow full imbibition.
2. Randomly select 100 seeds; longitudinally bisect each seed through the embryo center line. Place each half in separate petri dishes.
3. (Optional) Boil one half of the seeds for 5 min to inactivate enzymes as a negative control.
4. Add sufficient TTC Staining Solution (4%) to cover the seed halves. Incubate at 37 °C protected from light for 30–60 min.
5. Rinse 2–3 times with tap water and immediately evaluate embryo coloration.

(III) Brain-tissue staining

1. Equilibrate an appropriate volume of TTC Staining Solution (4%) to room temperature.
2. Collect mature, unopened fresh flowers; carefully remove petals and pistils.
3. Place pollen onto a glass slide, add 1–2 drops of TTC Staining Solution (4%), and cover with a coverslip.
4. Incubate at 35 °C for 15 min; observe under low-power microscope, counting five fields per slide.

Staining Results

Seed or pollen staining	Color
High viability	Red
Low viability	Pale red
Non-viable or sterile	Colorless

Myocardial or brain-tissue staining	Color
Normal myocardium or brain tissue	Red
Myocardial or cerebral infarct area	Pale

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Ischemic brain tissue	Intermediate between red and pale

Notes

1. TTC Stain (4%) is slightly irritating; handle with care.
2. Maintain brain integrity during dissection.
3. If staining is weak, prolong staining time appropriately.
4. Use the freshest samples possible; enzyme activity in normal myocardium and brain declines quickly—stain promptly.
5. Wear laboratory coat and disposable gloves for personal safety.
6. Use reagent soon after opening to ensure optimal performance.

This product is for research use only!