

## TTC Staining Kit, 0.5%

**Catalog No.: RA20110**

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### Basic Information

<b>Product name</b>	TTC Staining Kit, 0.5%
<b>Sizes</b>	100 mL
<b>Storage</b>	2-8 °C, keep away from light
<b>Shipping</b>	Shipped with ice pack
<b>Validity</b>	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 (0.5%) is mainly used for assessing the viability of seeds and pollen, whereas TTC Staining Solution (1%) is usually employed to observe myocardial infarction and necrotic areas in brain tissue, allowing earlier detection (3–6 h ahead of electron microscopy and 24 h ahead of light microscopy) of early infarcted tissue in experimental animal models. Features of this product: 1. Fresh tissue can be stained immediately without fixation, embedding, or other pre-treatment. 2. Short staining time, generally 10–30 min. 3. The solution can be reused; 10 mL can stain about 20 samples.

### Materials Required (Not Supplied)

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

### Experimental procedure

(I) Seed staining

1. Cut different viability seeds transversely and longitudinally along the embryo centre; keep half of

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each seed.

2. Incubate the halves in TTC Stain (0.5%) at 37 °C for 20 min protected from light.
3. Pour off the stain, rinse 2–3 times with tap water, and immediately observe embryo coloration.

### (II) Pollen staining

1. Prepare TTC solution.
2. Select fresh flowers about to open; carefully remove petals and pistils.
3. Place pollen material on a slide and add 1–2 drops of TTC.
4. Incubate at 35 °C for 15 min; observe under low-power microscope, counting five fields per slide.

### (III) Brain-tissue staining

1. Collect fresh brain sample (after anaesthesia with or without saline perfusion); freeze at –20 °C for 20–30 min to facilitate slicing.
2. Slice brain: 2–3 mm thickness for animals, 3–5 mm for human samples; obtain 4–5 consecutive sections. First cut: midpoint between frontal 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 Stain (0.5%) protected from light for 30–35 min.
4. Fix slices in 4% paraformaldehyde or 10 % neutral buffered formalin for 4–24 h.
5. Blot surface water and measure infarct area/volume with image-analysis systems such as IPP.

## 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
Ischemic brain tissue	Intermediate between red and pale

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### Notes

1. TTC Stain (0.5%) 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!**