

## TTC Staining Kit, 4%

**Catalog No.: RA20113**

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### Basic Information

<b>Product name</b>	TTC Staining Kit, 4%
<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 (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.

### Experimental procedure

#### (I) Brain Tissue Staining

1. Harvest fresh brain tissue (under anesthesia or after saline perfusion) and snap-freeze at  $-20\text{ }^{\circ}\text{C}$  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

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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
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### 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!**