

## 8. SOLUTIONS

The essential reagent is the tetrazolium staining solution. Other reagents may be used in facilitating seed conditioning, staining, or evaluation. These reagents include: gibberellic acid, pH buffers, hydrogen peroxide, mucilage hardeners, lactic acid, and glycerol. Material Safety Data Sheets (MSDS) produced by the chemical manufacturer are available for most reagents to alert consumers to toxicity, flammability, other hazards, and the proper disposal of used chemical solutions.

### 8.1 Tetrazolium solution

The recommended tetrazolium derivative is 2, 3, 5-triphenyl tetrazolium chloride (TTC). This salt is sold in powder form and is available from several chemical suppliers. Three sources are listed below:

MP Biomedicals, Inc. (formerly ICB)  
1263 S. Chillicothe Road, Aurora, OH 44202                      1-800-854-0530

Fisher Scientific Company  
1458 N. Lamon Avenue, Chicago, IL 60651                      1-800-766-7000

United States Biochemicals Corporation  
26201 Miles Road, Cleveland, OH 44128                      1-800-321-9322

The tetrazolium staining solution consists of the TTC and water. A buffer can be added to the TTC and water solution when there is need to correct a pH problem. Several concentrations of tetrazolium may be used with comparable results. For convenience, solutions of 1.0% and 0.1% TZ can be prepared and stored in cold (5° C) conditions. The TZ concentration used for the test is generally dependent on how the seed is prepared. A 1.0% solution is used for seeds that are not bisected through the embryo, while a 0.1% solution is used for seeds in which the embryo is bisected. Other low concentrations such as 0.2% and 0.5% are sometimes used.

To prepare a 1.0% solution of TZ, dissolve 1.0 gram of tetrazolium powder in 100 ml of distilled or tap water. A 0.1% solution is prepared by dissolving 0.1 gram of tetrazolium powder in 100 ml of water, or by mixing one part of 1.0% solution with nine parts of water.

Store all prepared TZ solutions in the dark or in amber- or brown-colored bottles to prevent deterioration from light. The TZ solution can be kept for several months if kept in a tightly capped container, in the dark, and refrigerated at 5° C.

The 2, 3, 5-triphenyl tetrazolium chloride MSDS describes and lists the known toxicological properties of the chemical. Although TZ is not considered to be a highly toxic or dangerous biological hazard (especially when diluted to liquid concentrations of 1.0% or less), there are proper protocols for the handling, usage, and disposal of the TZ solution (*APPENDIX*).

Laboratories must practice safety procedures to ensure the health and well-being of their employees and the safety of their lab facility. Latex and rubber gloves, safety eyewear, exhaust/fume hoods and chemical storage cabinets should all be part of the laboratory set-up. Labs should follow the MSDS guidelines and properly dispose of the chemical through incineration or chemical storage and transport methods. Disposal methods are dependent on state and local regulations. Safety and concern for employees and the environment are essential considerations.

## 8.2 Buffer solution

The pH for the aqueous TZ solution should be between 6.5 and 7.5 for optimum staining results. A pH meter or litmus paper is used to check the pH of the solution (low pH is acidic and high pH is basic). TZ may be made with water or a buffer solution. Hard or soft water may also affect the TZ solution and chemistry. If this is suspected, use distilled water or a buffer solution.

If making the solution with water, check the pH of the water before adding the TTC powder. If the pH is between 6.5 and 7.5, mix in the TTC powder at the desired concentration. Check the pH again. If the pH is out of the appropriate range, discard the solution and prepare a new batch of TZ using a buffer solution instead of water.

Many different chemical compounds can be used to make buffer solutions. In TZ testing, sodium phosphate and potassium phosphate are commonly used, prepared according to the following recipe:

**Solution 1** - Dissolve 9.078 grams  $\text{KH}_2\text{PO}_4$  in 1000 ml water.

**Solution 2** - Dissolve 9.472 grams  $\text{Na}_2\text{HPO}_4$  in 1000 ml water.

Mix two parts of solution 1 to three parts of solution 2. Then add TTC powder at the desired concentration. No further pH measurement or adjustment is necessary. The shelf life of buffer stock solution is approximately eight months when stored in a clean amber-colored bottle at 5° C.

## 8.3 Other solutions used in TZ testing

### 8.3.1 Lactic acid and glycerol

Lactic acid (85%) or glycerol can be used to remove pigmentation from the seed coats of certain small-seeded grasses, legumes, and other small-seeded species that are pierced or immersed whole. These solutions make the light-pigmented seed coats translucent, so that the stained embryo can be evaluated with an adequate light source and dissecting microscope. The average length of time for this 'clearing' treatment is one half hour. Clearing time is dependent on the tissue composition and pigmentation and may take several hours for some species. These solutions are easily obtained from most chemical companies and pharmacies. Lactic acid or glycerol can be used to preserve a stained sample for a limited time. (Lactophenol was once used because the phenol component helped preserve prepared seed; however, the toxicity of phenol compound makes this a hazardous option.)

### 8.3.2 Gibberellic acid ( $\text{GA}_3$ )

Gibberellic acid may be used to precondition dormant seed. (To make a 400 ppm solution, mix 400 mg  $\text{GA}_3$  in 1.0 liter of distilled  $\text{H}_2\text{O}$ .)

### 8.3.3 Hydrogen peroxide ( $\text{H}_2\text{O}_2$ )

3.0% concentration or less (0.5%) of  $\text{H}_2\text{O}_2$  is used as an oxygenating and bleaching agent during preconditioning (3.0% concentration can be purchased at pharmacies, then diluted with distilled water to obtain the desired concentration).