



---

## INT SUBSTRATE KIT FOR GLUCOSE OXIDASE

Catalog Number SK-3300

The INT substrate kit contains all of the reagents necessary to prepare a working solution of 2 (p-iodophenyl) – 3-(p-nitrophenyl) – 5-phenyl tetrazolium chloride for staining tissue sections. INT will yield a violet colored reaction product.

### DISPENSING REAGENTS:

For convenience the reagents are supplied in dropper bottles. When dispensing drops, hold the bottle in an inverted vertical position and squeeze gently. The drop volumes of each reagent in this kit are not necessarily the same. Only use the drop dispensers for preparing substrate solutions. To prevent evaporation, secure the opaque caps on the bottles when they are not in use. After completion of the staining procedure, the diluted working solution should be discarded, the container rinsed with distilled water and stored together with the stock reagents in the kit box.

### INSTRUCTIONS:

Immediately before use, add two drops each of reagents 1, 2, and 3 to 5 ml of 50 mM TRIS-HCl buffer, pH 9.5, in the substrate solution mixing bottle and mix well.

Incubate tissue sections with the substrate at room temperature in the dark until suitable staining develops. Development times should be determined by the investigator but generally 10 - 30 minutes provides good staining intensity.

Wash the sections for 5 minutes in water.

Counterstain and mount, using only an aqueous mounting medium. An aqueous mounting medium such as Immumount\* has been found to retain a more optimal signal intensity than glycerol/gelatin. (See reverse side for counterstain guidelines.)

### NOTES:

If at all possible, glass-distilled water should be used in the preparation of the substrate buffer.

The reagents should be stored at 4 °C and protected from light. Reagent 2 is very light sensitive and should be kept in the closed box whenever possible.

Elevation of the incubation temperature to 37 °C will shorten the development time or may permit the use of higher dilutions of primary antibody.

In the case of non-aqueous mounting, dehydration should be carried out rapidly.

**IMPORTANT:** Little is known about the toxicity and carcinogenicity of the substrate components. Care should be taken in the handling and disposing of all the reagents.

\* Trademark of Shandon Southern Instruments Inc.