

# DATA SHEET

## Elegance Red Substrate Pack

For Use with Alkaline Phosphatase Detection Kits  
(100mL/ 500 Tests)

Cat. No. **HK144-5KE**

Doc.No.932-HK144-5KE, Rev. No.1  
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### Intended Use

For In Vitro Diagnostic Use. Elegance Red Substrate Pack is intended for in vitro diagnostic use as chromogens for detection of specific antigen-antibody reactions in immunohistochemistry (IHC) and in situ hybridization (ISH).

### Summary and Explanation

Elegance Red produces a bright red reaction, which can be visualized with either bright field or fluorescent microscopy. The kit contains all of the reagents for an alkaline phosphate substrate working solution. This product provides crisp staining pattern and allows excellent visualization of cellular components. Elegance Red is alcohol/xylene insoluble, which can be mounted permanently in both non-aqueous and aqueous media.

### Principles of the Procedure

The demonstration of targets of interest in tissues and cells by immunostaining is a two-step process involving first, the binding of a primary antibody to the antigen of interest, and second, the detection and visualization of bound antibody by one of a variety of enzyme based chromogenic systems. The type of chromogenic substrate depends upon the type of enzyme used. Elegance Red can be used with Alkaline Phosphatase. *In situ* hybridization involves binding of specific nucleic acid probe that is labeled with a hapten. The label is subsequently detected using a primary antibody to the label followed by detection and visualization as above. Elegance Red is a substrate for Alkaline Phosphatase and offers high sensitivity for bright field or fluorescent microscopy. The bright red dye precipitate produces maximal contrast with blue counterstains and reproduces well by color photomicrography.

### Reagents Provided

Catalog No.	Substrate Packs	Format
HK145-2K	Red Reagent A	2 ml
HK146-2K	Red Reagent B	2ml
HK147-YAK	Red Buffer D	100ml
HK148-2K	Red Reagent C	2 ml

### Reagents Required but Not Supplied

All reagents required for IHC and ISH are not provided. See antibody and detection kit datasheet for complete set of reagents required for immunohistochemistry (IHC) or *in situ* hybridization (ISH) procedures.

### Storage and Handling

The reagents should be stored at 2- 4 °C and protected from light whenever possible. Occasionally a precipitate may form in some reagents upon prolonged storage. This will have no effect on the quality or intensity of the staining. Do not filter the reagents or working solution.

### Precautions

1. To prevent most non-specific staining, wash the sections thoroughly between steps in buffer for five minutes and rinse in water.
2. Use the Elegance Red substrate working solution within 05 minutes of preparation or declined sensitivity may occur.
3. Do not heat Elegance Red substrate kit components or working solution. Heating decreases staining sensitivity.
4. Avoid contact with the solution.
5. Do not put sodium azide in the buffer used for the Elegance Red working solution; it will prevent staining.
6. When using neural tissue inadequate staining may occur with Elegance Red for visualizing processes, fibers or terminals.

### Expected Results

Staining using the IHC and ISH systems should result in deposition of colored chromogen pigment at the site of specific interaction with minimal to no non-specific background, For Alkaline Phosphatase Kits, Elegance Red forms an intense red color.

### Staining Protocol

#### PREPERATION AND USE OF ELEGANCE RED CHROMOGEN

Prepare Elegance Red substrate working solution in a brown/black vial and use immediately.

1. To 5ml of HK147-YAK
2. Add 2 drops (~80µl) of HK145-2K and mix well.
2. Add 2 drops (~80µl) of HK146-2K and mix well.
3. Add 2 drops (~80µl) of HK148-2K and mix well.

\* 0.1% Tween 20 in the elegance red chromogen working solution is recommended, especially for use with antigen retrieved tissues.

4. Add the Elegance Red mixture to the tissue section. Incubate for 15-30 minutes. However, development time may vary due to the degree of specific antigen/antibody binding in the specimen.
5. Wash tissue section with buffer.
6. Counterstain in hematoxylin. Rinse in water.
7. Dehydrate, clear and mount in a permanent mounting medium. Or tap excess water from section and mount in an aqueous mounting media.

### Performance Characteristics

BioGenex has conducted studies to evaluate the performance of Elegance Red Substrate pack using several BioGenex IHC and ISH assays. Elegance Substrate Pack has shown reproducible and consistent results when used within a single run, between runs, between lots and wherever applicable between manual and automated runs. The product has been determined to be stable for the periods specified on the labels either by standard real time or accelerated testing methods. BioGenex ensures product quality through 100% quality control for all products released and through surveillance programs.

**EC|REP** Representative in the European Community; Mandatario nella Comunità Europea; Bevollmächtigter in der Europäischen Gemeinschaft; Representante autorizado en la Comunidad Europea

**IVD** In Vitro Diagnostic Medical Device; Dispositivo medico-diagnostico in vitro; In Vitro Diagnostikum; Producto sanitario para diagnóstico in vitro

**i** Consult Instructions for use; Consultare le istruzioni per l'uso; Gebrauchsanweisung beachten; Consulte las instrucciones de uso

**T** Temperature Limitation; Limiti di temperatura; Zulässiger Temperaturbereich ; Limite de temperatura

**M** Manufacturer; Fabbicante ;Hersteller; Fabricante