HistoMark®
X-Gal Substrate Set

Catalog No.	Size
54-13-00	100 mL

DESCRIPTION
The HistoMark X-Gal Substrate Set is designed for use with any probe labeled with *E. coli* β-galactosidase. The substrate produces a brilliant blue specific stain with no background color. The stain is permanent and insoluble in alcohols or xylene. Sufficient reagent is provided for approximately 200 slides.

FORM
This kit consists of the following:
1 x 100 mL Iron Buffer Reagent* (Catalog # 71-00-55)
1 x 3 mL X-Gal Substrate Solution** (Catalog # 71-00-56)

*Discard if microbial growth is evident.
**CAUTION: Contains N,N,-Dimethylformamide, suspected carcinogen, skin and eye irritant. Avoid contact with skin and eyes.

Sufficient material supplied to prepare 100 mL Substrate Solution.

STORAGE/STABILITY
Reagents are stable for a minimum of one year stored at 2 - 8°C.

REAGENTS REQUIRED BUT NOT INCLUDED
1. Primary antibodies.
2. β-Galactosidase-labeled antibodies or streptavidin.
3. Wash solutions used during various phases of the immunologic procedure, such as 100 mMol/L Tris-HCl, pH 7.6, Tris-buffered saline or PBS.

ACCESSORIES REQUIRED BUT NOT PROVIDED
1. Microscope.
2. Microscope Slides.
3. Cover Slips.
4. Test Tubes.
5. Mounting Media

BACKGROUND
β-galactosidase has been used in a variety of immunohistochemical techniques[1, 2, 3] and to detect β-galactosidase expressed in cells[4]. β-galactosidase isolated from *E. coli* has a pH optimum of 6 - 8, whereas the mammalian enzyme operates at a pH optimum of 3 – 5. This difference in pH allows antigen detection in mammalian tissue with no background due to endogenous enzyme activity.

PRINCIPLE
β-galactosidase catalyzes the hydrolysis of X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) to an indolyl alcohol, which then oxidizes to form an intense blue indigo stain. Bondi, et al[2], described a procedure using ferri-ferro-cyanide to accelerate the oxidation of the indoyl alcohol. HistoMark X-Gal Substrate Set is a modification of this procedure.

PROCEDURE
1. After the final step in the immunologic sequence (addition of β-galactosidase-labeled probe), rinse slides in wash buffer. Soak slides at least 5 minutes in a Coplin jar containing wash buffer.
2. Add 50 µL of X-GAL Substrate Solution to 2 mL of Iron Buffer Reagent. Mix thoroughly.
3. Shake off wash buffer from slides and wipe off excess surrounding tissues.
4. Completely cover tissues with reagent from Step 2.
5. Incubate for 15 – 30 minutes at room temperature or 37°C.
6. Rinse slides thoroughly in reagent quality water.
7. If desired, tissues may be counterstained for 2 – 4 minutes in Contrast Red, Catalog No. 71-00-05.
8. Dehydrate by rinsing briefly (ten dips) in 80% alcohol, 100% alcohol and xylene or xylene substitutes.
9. Mount in xylene-based mounting media.

NOTE: Aqueous mounting media may be used if the tissues are thoroughly air dried following steps 6 – 7.

RESULTS
Sites of enzyme activity appear brilliant blue. Nuclear material is pale red if section is counterstained with Contrast Red.

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NOTES

1. Always include positive, negative and reagent controls.
2. If color develops rapidly (less than two minutes), the primary antibody should be further diluted.

L-208-03

PRODUCT SAFETY AND HANDLING

See MSDS (Material Safety Data Sheet) for this product.

REFERENCES

1. Holzmann B. Johnson JP: A β-galactosidase-linked immunoassay for the analysis of antigens on individual cells. *J Immunol Methods* 60:359, 1983 (R147)
2. Bondi A, Chieregatti G, Eusebi V, Fulcheri E, Bussolati G: The use of β-galactosidase as a tracer in immunocytochemistry. *Histochemistry* 76:153, 1982 (R148)
3. van der Loos CM, Das PK, Houthoff HJ: An immuno-enzyme triple-staining method using both polyclonal and monoclonal antibodies from the same species. Application of combined direct, indirect and avidin-biotin complex (ABC)techniques. *J Histochem Cytochem* 35:1199, 1987 (R149)
4. Sanes JR, Rubenstein LR, Nicholas JF: Use of a recombinant retrovirus to study post-implantation cell lineage in mouse embryos. *EMBO J* 5:3133, 1986 (R165)

See KPL's catalog for a complete list of nucleic acid labeling and detection systems, antibodies, substrates and immunohistochemistry products.

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