BioGenex
Accelerating the pace of precision medicine

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EC REP

SUBSTRATE PACKS

Cat. No.	Substrate Packs	Format
HK542-XAKE	2- COMPONENT DAB PACK	1000 slides
HK182-5KE	FAST RED SUBSTRATE PACK	500 slides
HK183-5KE	NEW FUCHSIN SUBSTRATE PACK	400 Slides
HK139-06KE	ONE STEP AEC SOLUTION	60 slides
HK139-50KE	ONE STEP AEC SOLUTION	500slides
HL301-25K	BGX HRP Green Kit	250 slides
HL301-YAK	BGX HRP Green Kit	1000 slides
HL302-25K	BGX HRP Black Kit	250 slides
HL302-YAK	BGX HRP Black Kit	1000 slides

Intended Use

Substrate packs are 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

BioGenex provides several substrate pack options both for manual and automated systems for *in situ* detection using alkaline phospahatase and peroxidase enzymes. The kits are designed to reduce substrate incubation times and minimize exposure to chemical hazards. These substrate packs allow microscopic visualization of different cellular components depending on the marker used either by *in situ* hybridization (ISH) or immunohistochemistry (IHC).

Principal 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, e.g. AEC and DAB can be used with Horseradish Peroxidase; Fast Red and New Fuchsin can be used with Alkaline Phosphatase; Levamisole can be used with alkaline phosphatase substrate in the place of peroxide block to block endogenous phosphatase staining. *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.

DAB (diaminobenzidine) substrate offers the greatest sensitivity of all the Horseradish Peroxidase colorimetric chromogens. The insoluble, permanent brown, black or green precipitate has a high-contrast in photographs. In addition, the sensitivity can be enhanced by carrying out the reaction in the presence of nickel or cobalt chloride and/or by examining slides by reflection interference microscopy (10-100x sensitivity).

AEC (aminoethyl carbazole) is a colorimetric substrate for Horseradish Peroxidase. The bright brick-red reaction product is insoluble in water, but soluble in alcohol and xylene. The AEC substrate is suitable for immunohistochemistry (IHC), in situ hybridization (ISH), and membrane blotting applications. For IHC and ISH, the AEC substrate is compatible with aqueous mounting media.

Permanent Fast Red (4-chloro-2-methyl-benzenediazonium salt) is a substrate for Alkaline Phosphatase and offers high sensitivity for light microscopic observations. The bright red dye precipitate produces maximal contrast with blue counterstains and reproduces well by color photomicrography. The reaction product is insoluble in water, and alcohol.

New Fuchsin

New Fuchsin, also known as Magenta III, produces an intense red/fuschia precipitate in the presence of alkaline phosphatase. It is not soluble in xylenes and alcohols; therefore it may be permanently mounted.

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

Store all reagents at 2-8°C. Do not use after expiration dates as indicated on the reagent labels.

Staining Procedure

Refer to detection system manual for relevant staining protocols.

Kit Component and Preparation & Use of Substrate Solution

HK542-XAKE – (2- COMPONENT DAB PACK)

1. DAB Chromogen-HK124-05K- (5ml)
2. Stable DAB Buffer- HK520-YAK-(100ml)

Preparation - Add 1 drop (~40ul) of Liquid DAB Chromogen to 1 ml ready-to-use Substrate Buffer. Mix well before using. This solution remains stable at room temperature (20-26°C) up to 6 hours.

Use- Apply upto 100ul of DAB working solution on tissue and incubate for 5-10 min at room tempratue. Therafter wash with running DI H_20/Tap water for 5 min or 4-5 washes 30 sec each wash and do counterstaining and dehydration. Dry the slide and mount with mounting media.

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HK139-06KE & HK139-50KE- (ONE STEP AEC SOLUTION)

1. One Step AEC Solution- 6ml and 50ml

Preparation – It is ready to use reagent

Use- Apply upto 100ul of AEC one step solution on tissue and incubate for 5-10 min at room temerature. Therafter wash with running DI H_20/Tap water for 5 min or 4-5 washes 30 sec each wash and do counterstaining and dehydration. Dry the slide and mount with aquous mounting media.

HK182-5KE – (Fast Red Substrate Pack)

1. Fast Red Chromogen-HK181-1K- (1ml)
2. Fast Red Buffer-HK180-50K- (50ml)

Preparation - Add 20ul of Fast Red Chromogen to 1 ml ready-to-use Fast Red Buffer. Mix well before using. This solution remains stable at room temperature (20-26°C) up to 6 hours.

Use- Apply upto 100ul of Fast Red working solution on tissue and incubate for 15-20 min at room tempratue. Therafter wash with running DI H_20/Tap water for 5 min or 4-5 washes 30 sec each wash and do counterstaining and dehydration. Dry the slide and mount with mounting media.

HK183-5KE – (NEW FUCHSIN SUBSTRATE PACK)

1. New Fuchsin Substrate- HK187-5K- (5ml)
2. New Fuchsin Tris Buffer- HK184-5K- (40ml)
3. New Fuchsin Chromogen-HK185-5K- (2.5ml)
4. New Fuchsin Activator-HK186-5K– (2.5ml)

Preparation- Combine 50 µl (1drop) of New Fuchsin Chromogen Solution and 50µl (1 drop) of New Fuchsin Activator Solution in the bottom of the mixing vial. This is a critical step for successful color development. Mix reagents thoroughly by repeated gentle pipetting. Empty contents of one vial of Tris Buffer into Chromogen-Activator mixture prepared above and mix well. Add 400 µl (8-10 drops) of Substrate Solution to the solution prepared above, and mix well. NOTE: If endogenous alkaline phosphatase is suspected, add levamisole (HK113-5K) at a concentration of 0.6 mg/ml to the Substrate at this time to inhibit the alkaline phosphatase activity. Prepare fresh substrate immediately prior to use.

Use- Apply upto 100ul of New Fuchsinworking solution on tissue and incubate for 15-40 min at room tempratue. Therafter wash with running DI H_20/Tap water for 5 min or 4-5 washes 30 sec each wash and do counterstaining and dehydration. Dry the slide and mount with mounting media.

HL301-25K & HL-301-YAK (BGX HRP Green Kit)

1. BGX HRP Green Chromogen- HL304-01K-(1ml), HL304-04K(4ml)
2. BGX HRP Green Buffer- HL303-25K-(25ml), HL303-YAK (100ml)

Preparation: Add 1 drop (~40ul) of BGX HRP Green Chromogen to 1 ml ready-to-use BGX HRP Green Buffer. Mix well before using. This solution remains stable at room temperature (20-26°C) up to 6 hours.

Use- Apply upto 100ul of Green chromogen working solution on tissue and incubate for 5-10 min at room tempratue. Therafter wash with running DI H_20/Tap water for 5 min or 4-5 washes 30 sec each wash and do counterstaining and dehydration. Dry the slide and mount with mounting media.

HL302-25K & HL-302-YAK (BGX HRP Black Kit)

1. BGX HRP Black Chromogen- HL306-02K -(1ml), HL306-08K(4ml)
2. BGX HRP Black Buffer- HL305-25K-(25ml), HL305-YAK (100ml)

Preparation: Add 2 drop (~80ul) of BGX HRP Black Chromogen to 1 ml ready-to-use BGX HRP Black Buffer. Mix well before using. This solution remains stable at room temperature (20-26°C) up to 6 hours.

Use- Apply upto 100ul of Black chromogen working solution on tissue and incubate for 5-10 min at room tempratue. Therafter wash with running DI H_20/Tap water for 5 min or 4-5 washes 30 sec each wash and do counterstaining and dehydration. Dry the slide and mount with mounting media.

Precautions

HK542-XAKE, HL302:  Rep 2;R61.  Xn;R20/21.  Xi;R36 = May cause harm to the unborn child.Harmful by inhalation and in contact with skin.Irritating to eyes. S25 S26 S38 S45 S53 S36/37/39 S60 P11 = Avoid contact with eyes.In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.In case of insufficient ventilation, wear suitable respiratory equipment.In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).Avoid exposure - obtain special instructions before use.Wear suitable protective clothing, gloves and eye/face protection.This material and its container must be disposed of as hazardous waste.

HK183-5KE, HK139, HK182,HL301:  T;R23-25.  C;R35.  Xi;R37 = Toxic by inhalation.Toxic in contact with skin.Causes severe burns.Irritating to respiratory system. S26 S38 S45 S24/25 S36/37/39 S60 = In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.In case of insufficient ventilation, wear suitable respiratory equipment.In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).Avoid contact with skin and eyes.Wear suitable protective clothing, gloves and eye/face protection.This material and its container must be disposed of as hazardous waste.

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 AEC Kits: AEC (3-amino-9-ethylcarbazole) forms a brick red end product.
For DAB Kits: DAB (3,3'-diaminobenzidine) forms a brown,black or green end product.
For Alkaline Phosphatase Kits: Fast Red forms an intense red color.
For New Fuchsin Kits: New Fuchsin produces an intense red/fuschia precipitate.

Limitations

Super Sensitive™ Detection kits demonstrate antigens that survive tissue fixation, embedding and sectioning. Correct treatment of tissues prior to fixation and embedding, while less critical for BioGenex Super Sensitive™ Reagents, is still important for obtaining optimal results. Inconsistent results may be due to variation in fixation and embedding methods employed by different laboratories, as well as from inherent variations in tissue. The results from immunostaining must be correlated with other laboratory findings and the relevant controls. An internal tissue processing control (e.g. vimentin) may be used to reveal errors in tissue processing. Use of BioGenex Antigen Retrieval pretreatment technique may permit recovery of antigenicity in formalin-fixed tissue. Please call BioGenex for more information on these products and their use in the standardization of immunostaining results.
The clinical interpretation of any positive staining or its absence should be evaluated within the context of clinical presentation, morphology and other histopathological criteria. The clinical interpretation of any positive staining or its absence should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests. It is the responsibility of a qualified pathologist who is familiar with the proper use of IHC antibodies, reagents and methods to interpret the stained preparation. Staining is to be performed in a certified licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase. (Omata, et al. 1980)
Normal/ non-immune sera from the same animal source as secondary antisera used in blocking steps may cause false-negative or false-positive results due to autoantibodies or natural antibodies.
False-positive results may be seen due to non-immunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome C), or endogenous biotin (e.g., liver, breast, brain, kidney) depending on the type of immunostain used. (Nadji & Morales, 1983).

Quality Control

Refer to the appropriate detection system package inserts for guidance on general quality control procedures.

Troubleshooting

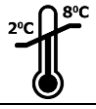
Refer to the troubleshooting section in the package inserts of BioGenex Super Sensitive Detection Systems (or other equivalent detection systems) for remedial actions on detection system related issues, or contact BioGenex Technical Service Department at (925) 275-0550 to report unusual staining.

Performance Characteristics

BioGenex has conducted studies to evaluate the performance of all its substrate packs using several BioGenex IHC and ISH assays. BioGenex substrate packs have shown reproducible and consistent results when used within a single run, between runs, between lots and wherever applicable between manual and automated runs. The products have 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.

References

1. Sheehan DC and Hrapchak BB. Theory and Practice of Histotechnology. St. Louis: C.V. Mosby Co. 1980.
2. Kiernan JA. Histological and Histochemical Methods: Theory and Practice. New York: Pergamon Press 1981.
3. Nadji M, Morales AR. Immunoperoxidase, part I: the techniques and its pitfalls. Lab Med 1983; 14:767-770.
4. Omata M, Liew CT, Ashcavai M, Peters RL. Nonimmunologic binding of horseradish peroxidase to hepatitis B surface antigen. A possible source of error in immuno histochemistry. Am J ClinPathol1980 ;73(5):626-632.

	Temperature Limitation	IVD	In Vitro Diagnostic Medical Device
	Use By Date	LOT	Batch Code
	Non-Sterile		Consult Instructions for Use
EC REP	Representative in the European Community		Manufacturer