



IHC Link-Label

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Presented by: BioGenex Technical Support

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BioGenex Immunohistochemistry (IHC) Quick Guide#

Super Sensitive™ Link-Label kits



Overnight, 50°C
then
15-30 min, 60-80°C
Before staining

EZ-DeWax 3 x 3 min, RT

For 95% of BioGenex antibodies
Use EZ-AR -2 Elegance- 20 min, 95°C

See Catalog for antibody-specific
recommendation

5-10 min, RT

5-10 min, RT



For >90% of BioGenex
antibodies: 30 min @ RT

10-20 min, RT

20-30 min, RT

5-10 min, RT

1-3 min, RT

Super Mount® Medium
Or
Xmount (for Automation)

**Example of Cytokeratin 18
Staining in FFPE tissue**

See Datasheet for
antibody-specific
Incubation conditions

For use with FFPE tissue slides. Washing steps omitted for simplicity - for full protocol see Link-Label kit datasheet.
Optimization may be required due to variation in tissues/processing.

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1. Baking – Heating the tissue slide helps remove humidity and promote attachment of tissue to slide. It also helps wax removal in the following De-Wax step. Depending on tissue type and thickness, incubation temperature and time may vary.

2. DeWax – BioGenex EZ-DeWax Solution (HK585-5K & HK584-5K) is a “one-step” product that simultaneously enables the removal of paraffin and allows rehydration of the tissue with a single xylene-free, non-toxic reagent.

3. Antigen-Retrieval - BioGenex is the inventor of Antigen Retrieval enabling technology. Antigen-retrieval step breaks the formalin induced cross-linking bonds between epitopes and unrelated proteins, thereby allowing better penetration of antibody and accessibility of epitopes. BioGenex solutions facilitate the production of highly reproducible and superior

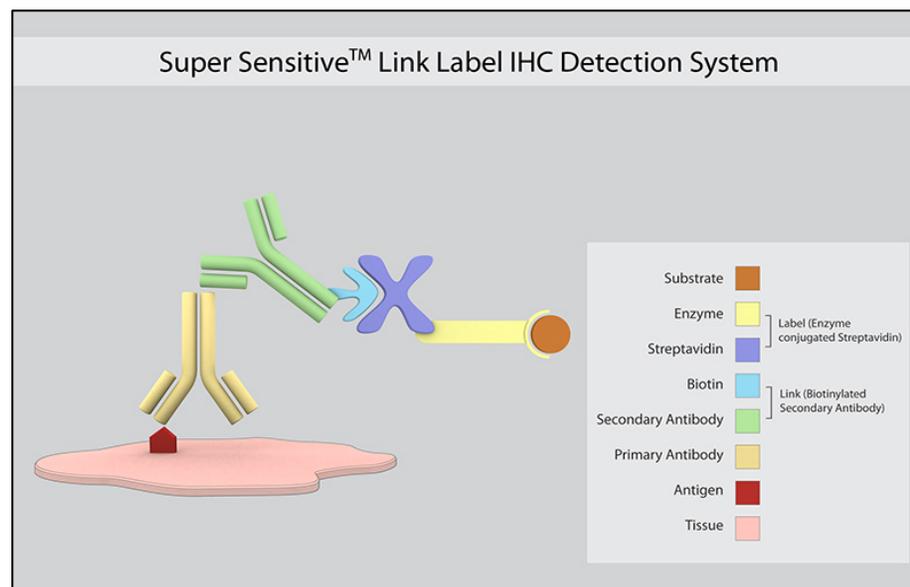
quality stains in a considerably short period of time without compromising the morphology and antigenicity of the tissue. The EZ-AR2-Elegance Solution advanced formulation (HX032-YCD & HK547-XAK) has unique superheating properties and was validated to give the best staining with 95% of BioGenex Antibodies (see datasheet for Ab-specific recommendation).

4. Peroxide Block (HK111-50K) – Hydrogen peroxide is used to inactivate endogenous peroxidase when Horseradish Peroxidase is used for staining. This eliminates background, especially in tissues such as red blood cells, kidney, and liver.

5. Power Block (HK083-50K) – This reagent contains buffer, casein and preservative and reduces background staining due to non-specific binding of antibodies. It is a potent blocker and should not remain on the tissue more than ten minutes.

6. Primary Antibody - BioGenex offers >400 antibodies for IHC staining. Antibodies are available in ready-to-use (RTU) or concentrated formats and are for manual or automated staining. BioGenex antibodies are optimized to provide maximum signal with minimum background.

7. Link-Label – A classic system based on the highly specific and sensitive streptavidin-biotin interaction (e.g. QA900-9LE, QP900-9L, QD470-YCXE).



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7. (cont.) LINK reagent is a mix of anti-mouse and anti-rabbit IgGs conjugated to multiple biotin molecules. The LINK antibodies detect and bind to the antigen-bound primary-antibody.

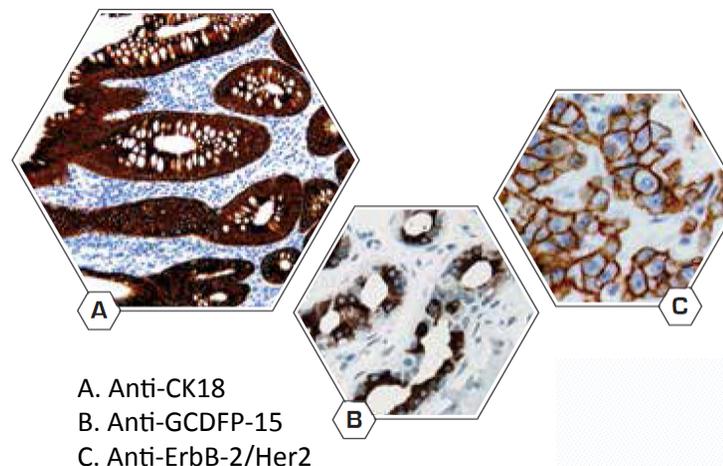
LABEL reagent is Streptavidin conjugated with the enzyme Horseradish peroxidase (HRP) (kits with Alkaline Phosphatase (AP) are also available). Streptavidin binds extremely strongly and irreversibly to the biotin residues on the secondary antibody resulting in the addition of multiple enzymes to the primary antibody complex.

8. DAB (HK542-XAKE) – DAB (3,3'-diaminobenzidine tetrahydrochloride) is a chromogenic peroxidase substrate. HRP catalyzes the oxidation of DAB to form a brown precipitate which stains the tissue in close proximity to the HRP conjugated antibody. DAB is insoluble in alcohol, xylene or water and therefore is compatible with most clearing agents and mounting media.

9. Hematoxylin (HK100-5K, HK100-9K) – (BioGenex Mayer's hematoxylin is a blue nuclear counterstain that makes the stained tissues more easily visible. It is alcohol and xylene insoluble and therefore compatible with most clearing agents and mounting media.

10. Mounting- Mounting seals the biological specimens and is an essential step before microscopic evaluation. BioGenex SuperMount® Permanent Mounting Medium (HK079-5K, HK079-7K) is a polymer based aqueous mounting media that does not require the use of a coverslip. XMount™ Mounting Medium (HX035-YCD, HX035-10X) is a permanent mounting medium that has been optimized for use with BioGenex Xmatrix automation platform. BioGenex also offers Aqueous Mounting Medium (HK099-5K) - a glycerol-based mounting medium that require the use of a coverslip. It is intended for short-term specimen storage and is compatible with most chromogens and counterstains.

11. Washing steps – Typically 2-3 washes between each step are required. BioGenex Super Sensitive™ Wash Buffer (HK583-5K) is a phosphate buffered saline (PH 7.4) with surfactant and is the best choice in order to ensure optimal staining. The buffer promotes even spreading of antibodies and other reagents to avoid inconsistent results.



Super Sensitive™ IHC Detection System kits Composition

Detection Systems - LINK LABEL (For mouse & rabbit antibodies)

For manual use (drop bottles) For i6000 Automation (Barcode labeled)

SKU	Size	Multi-Link	Label	DAB buffer	DAB Chromogen	Peroxide block	Power block	Hematox	Negative ctrl IgG
QA900-9LE	500 test	50 ml	AP 50 ml	-	-	-	-	-	-
QP900-9LE	500 test	50 ml	HRP 50 ml	-	-	-	-	-	-
QP300-XAKE	1000	100 ml	HRP 100 ml	-	-	-	-	-	-
LP000-ULE	1000+	5 ml (Conc.)	HRP 5 ml (Conc.)	-	-	-	-	-	-
LA000-ULE	1000+	5 ml (Conc.)	AP 5 ml (Conc.)	-	-	-	-	-	-
QD470-YCXE - i6000	200 test	20 ml	HRP 20 ml	5x20 ml	4 ml	20 ML	20 ml	20 ml	-

IHC Detection Systems - Links / Labels items-Manual

Product	5 mL ^(Conc.)	6 ml ^(RTU)	50 ml ^(RTU)
SS AP label	HK321-UK	HK331-5K	HK331-9K
SS Goat Link	HK327-UG	HK337-5G	N/A
SS HRP label	HK320-UK	HK330-5K	HK330-9K
SS Mouse Link	HK325-UM	HK335-5M	HK335-9M
SS Multi Link (ANTI-mouse & rabbit)	N/A	HK340-5K	HK340-9K
SS Rabbit Link	HK326-UR	HK336-5R	HK336-9R
SS Rat Link	N/A	HK338-5T	N/A
Conc. Multi Link (ANTI-Mouse, Guinea Pig, Rat and Rabbit)	HK268-UK	N/A	N/A

Thank You

Please visit www.biogenex.com for more details on our product portfolio

