Versatility of Detection Method

HisDetector Nickel Conjugates are offered as stand-alone reagents or combined with KPL's optimized reagents and substrates to provide complete Western blot kits. Figures 5 and 6 below illustrate comparable sensitivity and low background for all HisDetector Western Blot Kits. Choose the kit that best fits your needs.

KPL HisDetector Western Blot Kits, HRP and AP, Chemiluminescent and Colorimetric, Offer High Sensitivity and Low Background.

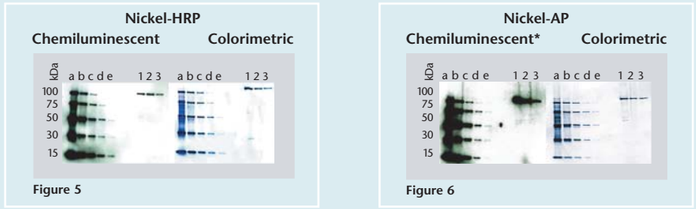

Figure 5

Figure 6

Figures 5 and 6: Blots were developed using the HisDetector Western Blot Kit procedure. Three-fold serial dilutions of His-tagged protein ladder (lanes a-e) and *E. coli* crude lysate containing His-tagged *β*-gal (lanes 1-3) starting at 50 - 75 ng and 200 ng, respectively, were used. The protein samples were separated over a 4 - 20% SDS-PAGE gel and transferred onto nitrocellulose membrane. Figure 5 membranes were treated with either LumiGLO® (chemiluminescent) and exposed to film for 10 minutes, or TMB (colorimetric). Figure 6 membranes were treated with either PhosphaGLO™ (chemiluminescent) or BCIP/NBT (colorimetric).

*PhosphaGLO AP Substrate is offered separately.

Ordering Information

Catalog#	Description	Size
	HisDetector Western Blotting Kits Each kit includes Nickel-HRP or Nickel-AP, Blocking Solution, Wash Solution Concentrate, and substrate.	
24-00-01	HisDetector Western Blot Kit, HRP Colorimetric	40 blots
24-00-02	HisDetector Western Blot Kit, HRP Chemiluminescent	40 blots
25-00-01	HisDetector Western Blot Kit, AP Colorimetric	40 blots
	HisDetector Nickel Conjugates	
24-01-01	HisDetector Nickel-HRP Conjugate Contents: HisDetector Nickel HRP HisDetector Nickel-HRP Dilution Buffer	 0.1 mL 1.0 mL
25-01-01	HisDetector Nickel-AP Conjugate Contents: HisDetector Nickel-AP Detector™ Block Solution	 1.0 mL 120 mL

To order or for more information, contact us at 800.638.3167 / 301.948.7755, fax 301.948.0169 or visit us at www.kpl.com.

ML307-02

HisDetector™

FAQs

What length of His-tag can be detected with HisDetector Nickel Conjugates?

HisDetector nickel conjugates will bind to all sizes of histidine residues. Four to ten consecutive histidine residues provide optimal levels of binding residues.

Do I need to use special block or wash solutions with HisDetector Nickel Conjugates?

Yes, specialized wash and block solutions are recommended. These reagents are provided in our Western Blot Kits. (See Table below.)

Feature	Nickel-AP	Nickel-HRP
Block	Detector Block	BSA
Wash	1X TBST	
Avoid	Avoid metal chelators such as EDTA and imidiazole; sodium azide (HRP only)	

Can HisDetector Nickel Conjugates be used with colorimetric and chemi-luminescent detection systems?

HisDetector Nickel Conjugates have been optimized for both colorimetric and chemiluminescent detection.

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www.kpl.com

ISO 9001:2008 Registered

KPL
SEE MORE with KPL!

HisDetector™

Fly through your assay!

Detect His-tagged proteins faster with KPL HisDetector™ Nickel Conjugates and Western Blot Kits.

HisDetector™ Nickel Conjugates and Western Blot Kits provide a fast and reliable method for directly detecting His-tagged recombinant proteins. Detection is based on nickel-NTA which binds with high affinity to histidine residues. They enable simple, direct detection of His-tagged proteins.

Using unique linker technology, KPL has coupled nickel-nitrilotriacetic acid (NTA) to horseradish peroxidase (HRP) and alkaline phosphatase (AP). These nickel conjugates form the basis of the HisDetector products. They are offered with three detection systems to provide high sensitivity without background.

Save Two Hours with Direct Detection

HisDetector nickel conjugates provide direct detection of recombinant proteins without the use of primary and secondary antibodies. Save two hours in a Western blot procedure compared to protocols using anti-His and secondary antibodies.

Simplify Assay Development with Optimized Kits

HisDetector Western Blot Kits simplify assay development. All the reagents necessary for rapid detection of His-tagged proteins are contained in convenient kits. No antibody or reagent optimization is required.

Choose from a Variety of Detection Formats

The HisDetector product line offers versatility. Kits are available using either HRP or AP nickel conjugates and your choice of chemiluminescent or colorimetric detection. No need to purchase new equipment - choose the kit that best fits your laboratory.

Fly through your assay. Detect His-tagged proteins without the background seen in other protocols. Save time *and* SEE MORE with KPL!

KPL
SEE MORE with KPL!

HisDetector™

HisDetector Nickel Conjugates and Western Blot Kits

KPL's HisDetector Nickel-HRP and Nickel-AP Conjugates and Western Blot Kits provide a rapid means for detection of His-tagged recombinant proteins through the use of nickel-NTA covalently conjugated to the reporter enzymes HRP or AP. NTA is a metal chelator that occupies four of the six binding sites in the coordination sphere of nickel, leaving two sites free on the nickel to bind with high affinity to His-tags. (Figure 1).

One-Step Procedure for Fast Results

Nickel-NTA enzyme conjugates bind directly to the His-tag, saving you several hours compared to the multi-step anti-His/secondary antibody alternative in a Western blot protocol. Figure 2 below compares direct detection using nickel conjugates with detection using antibody reagents. HisDetector nickel conjugates can also be used in other applications, such as ELISA and immunohistology.

His-tagged Protein Interaction with Ni-NTA Conjugated to HRP or AP

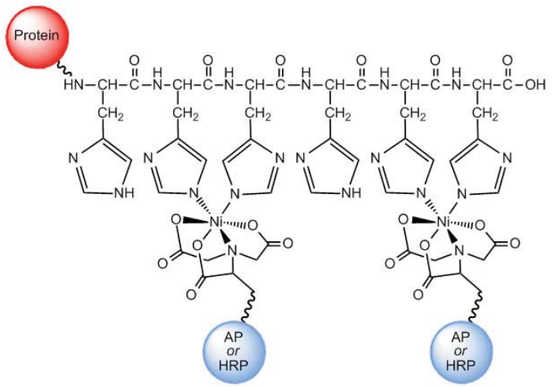

Figure1. Interaction of polyhistidine sequence with Ni-NTA conjugated to HRP or AP.

Western Blot Detection of His-Tagged Proteins

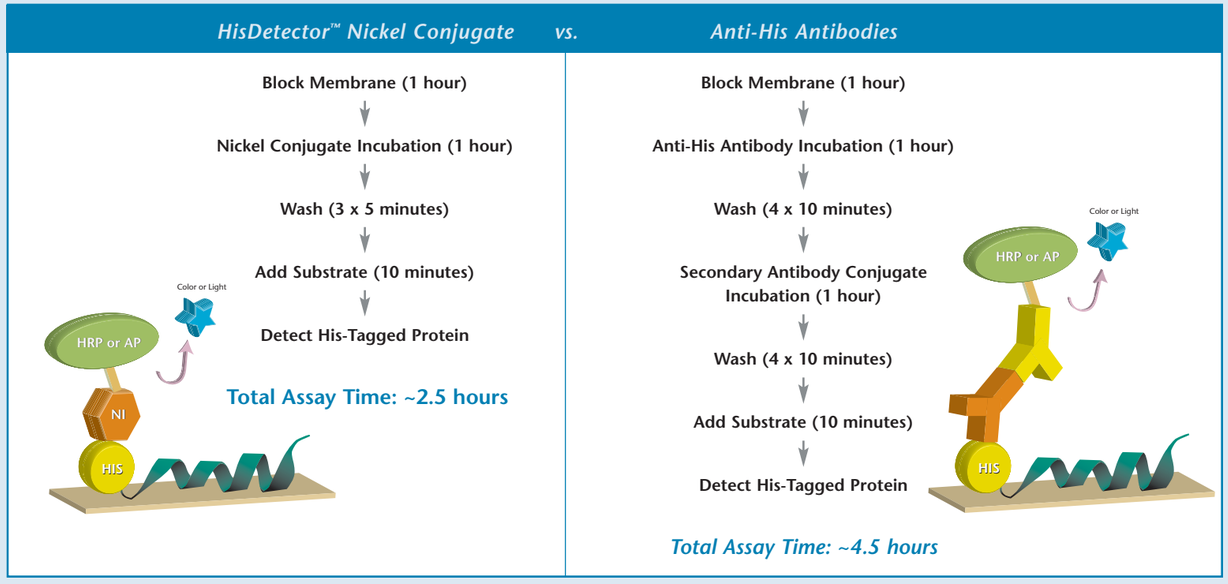

Figure 2

Unsurpassed Performance

HisDetector Nickel Conjugates and kits offer the best combination of high sensitivity and low background when compared to other single-step His-tagged protein detection methods. As seen in Figure 3 and 4, picogram range detection of His-tagged protein is possible with HisDetector HRP Conjugate in both chemiluminescent and colorimetric assays. In both cases, a strong, specific signal is produced while background remains very low.

In contrast, alternative single-step methods result in lower sensitivity (Figure 3, B; Figure 4, B and C) in both chemiluminescent and colorimetric detection protocols. High sensitivity is observed with certain products, but high background significantly reduces readibility of the blot. (Figure 3, C). In addition, alternative protocols require more than three hours to perform. See below for a comparison of single-step His-tagged protein detection methods.

Comparison of Single-Step His-tagged Protein Detection Methods

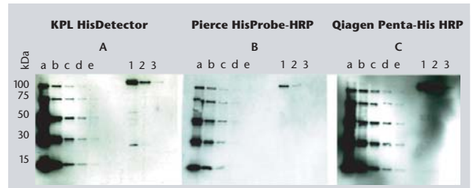

Figure 3: Chemiluminescent Detection

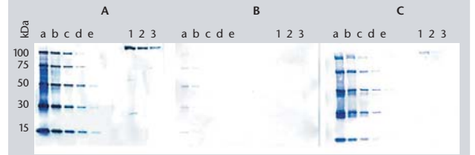

Figure 4: Colorimetric Detection

Detection of His-tagged protein ladder (lanes a - e) and a recombinant His-tagged ß-gal (lanes 1-3).

KPL HisDetector Nickel-HRP Conjugate was compared with two other HRP-labeled, single step detection methods for His-tagged proteins to demonstrate relative sensitivity and background in chemiluminescent and colorimetric Western blotting assays.

Three-fold serial dilutions of His-tagged protein ladder (lanes a-e) and *E.coli* crude lysate containing His-tagged ß-gal (lanes 1-3) starting at 50 - 75 ng and 200 ng, respectively, were used. The protein samples were separated over a 4 - 20% SDS-PAGE gel and transferred onto nitrocellulose membrane. Conjugate incubation, blocking and washing steps were performed according to the manufacturers' recommended protocols. Membranes were treated with either LumiGLO® Substrate (Figure 3) and exposed to film for 10 minutes, or with TMB Membrane Substrate (Figure 4).

Comparison of HisDetector™ with Other Single-Step Detection Methods in Western Blotting

Feature	HisDetector Kits	Pierce HisProbe-HRP Kit	Qiagen Penta-His HRP Conjugate Kit
Type of Ligand	Nickel Conjugate	Nickel Conjugate	HRP Anti-His Antibody
Assay Time	2.5 hours	>3 hours	>3 hours
Sensitivity	Picogram	Nanogram	Picogram
Specificity	High	High	Low
Background	Low	Low	High
Complete Blotting Kit	Yes	Yes	No
Kit Detection Format:			
HRP Chemiluminescent	Yes	Yes	No
AP Chemiluminescent	No*	No	No
HRP Colorimetric	Yes	No	No
AP Colorimetric	Yes	No	No

Table 1

*Nickel-AP Conjugate is optimized for use with PhosphaGLO™ AP Substrate (KPL catalog #55-60-04) and can be purchased separately.

SEE MORE with KPL!
Get specific signal without background using HisDetector products and SEE MORE!

Pictured on front and above: Little Bee Eaters