

# Rapid Detection of His-tagged Fusion Proteins in Western Blots with KPL's HisDetector™ Nickel-NTA Enzyme Conjugates



www.kpl.com

Joshua D. Levin, Ph.D., Danielle Russell, Gordana Pajkovic and Mekbib Astatke, Ph.D.

KPL, Inc., 910 Clopper Road, Gaithersburg, Maryland 20878

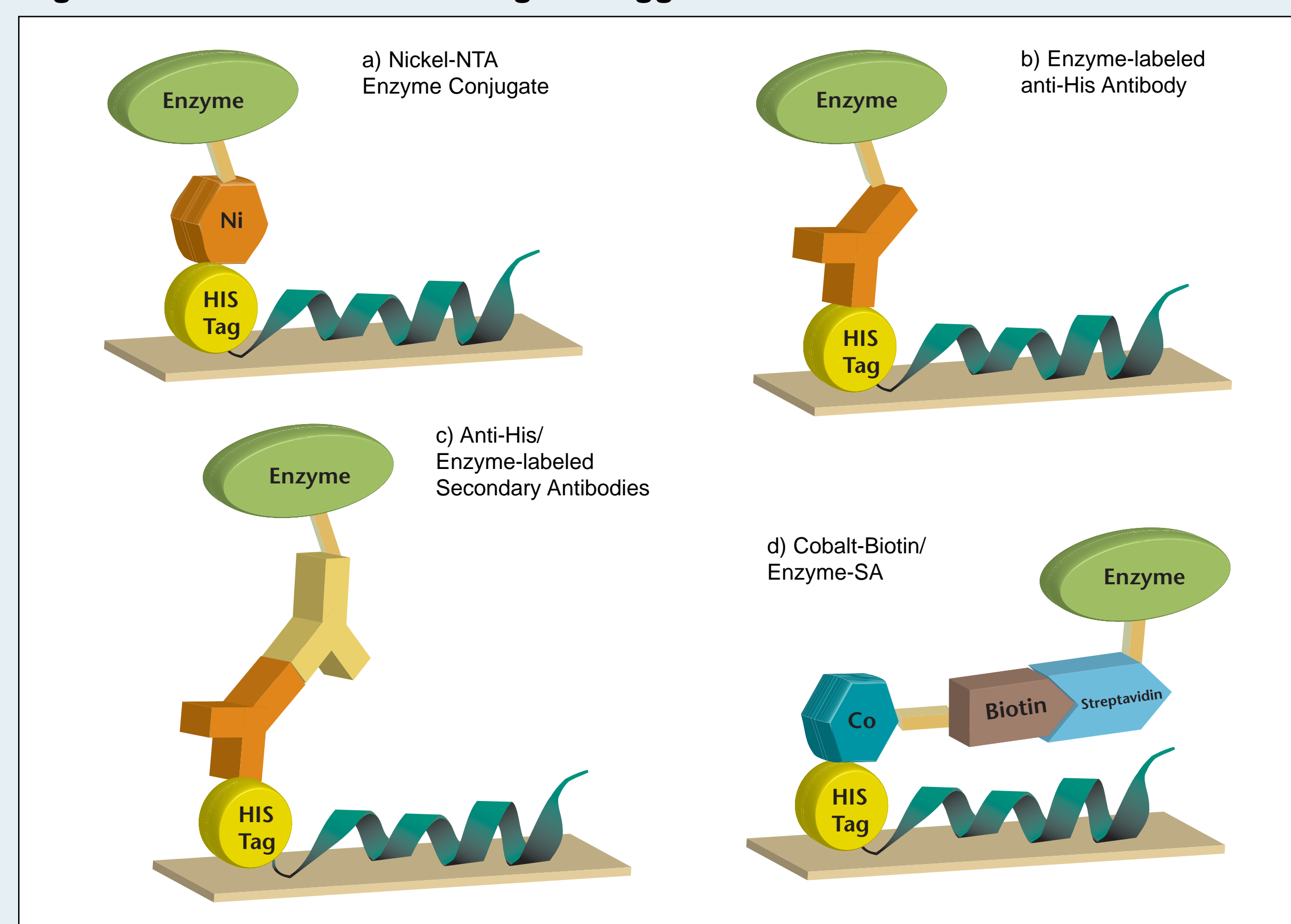
## INTRODUCTION

Expression and purification of His-tagged proteins can be monitored by one-step or two-step detection methods:

- One-step methods may use an enzyme-labeled primary anti-His antibody (Figure 1b) or an enzyme-labeled nickel-nitrilotriacetic acid (NTA) conjugate (Figure 1a).
- The most common two-step method (Figure 1c) uses a combination of an anti-His primary antibody and an enzyme-labeled secondary antibody.
- An alternate two-step method uses biotin-labeled cobalt and enzyme-labeled streptavidin (Figure 1d).

We compare the performance of a Ni-NTA conjugate with that of an anti-His conjugate for detection of his-tagged proteins in Western blots. We also compare the time and number of steps for commonly used one- and two-step methods.

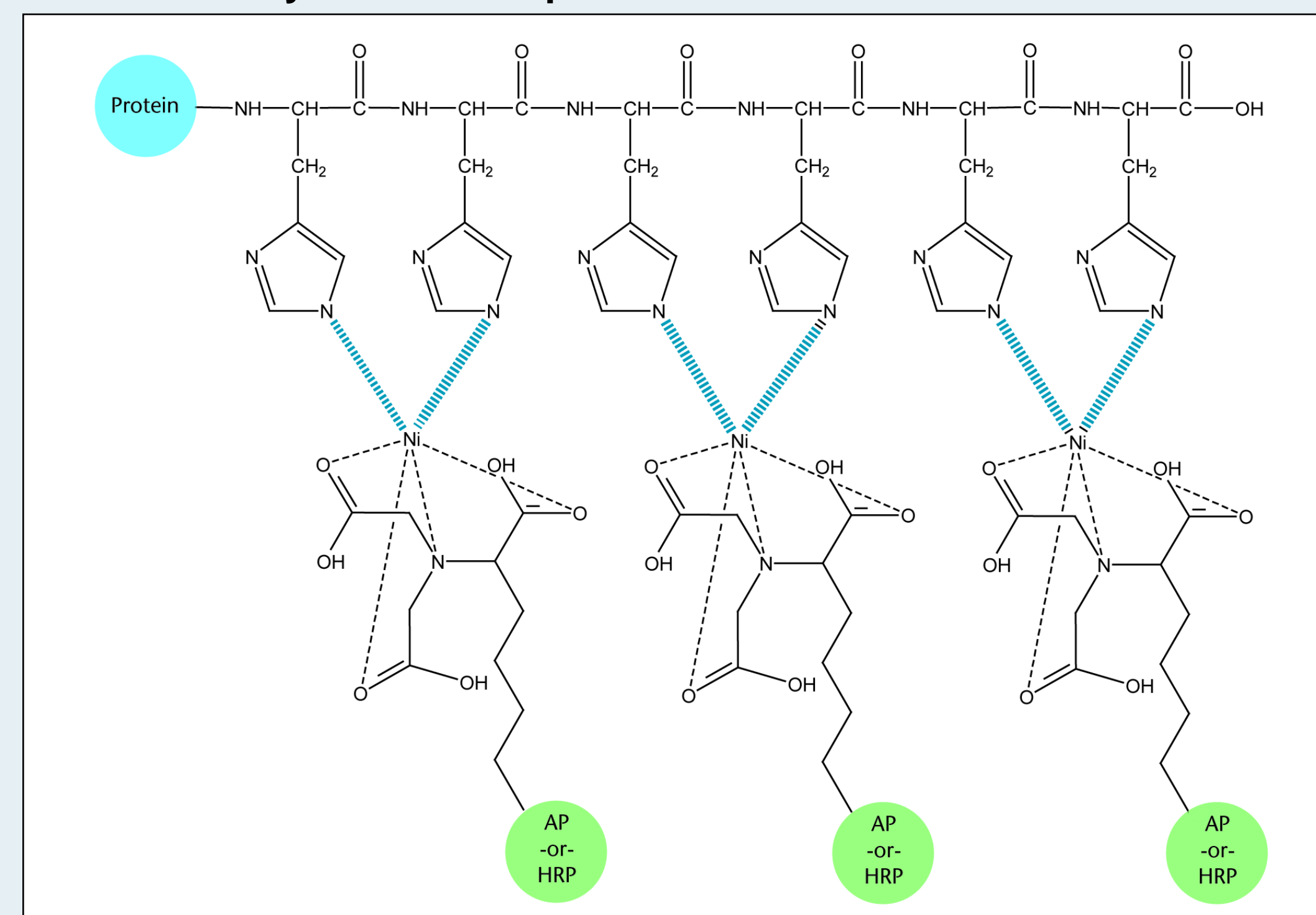
Figure 1. Methods for Detecting His-tagged Proteins



The interaction between a 6X His-tag and a Ni-NTA enzyme conjugate is depicted in Figure 2:

- Alkaline Phosphatase (AP) or Horseradish Peroxidase (HRP) is linked to a NTA group which conjugates nickel via four coordination sites.
- The NTA is linked to the enzyme via proprietary linkage technology (patent pending).
- The two unoccupied coordination sites on the nickel ion bind to the His-tag.

Figure 2: Interaction of Ni-NTA-Enzyme Conjugates with a Polyhistidine Sequence



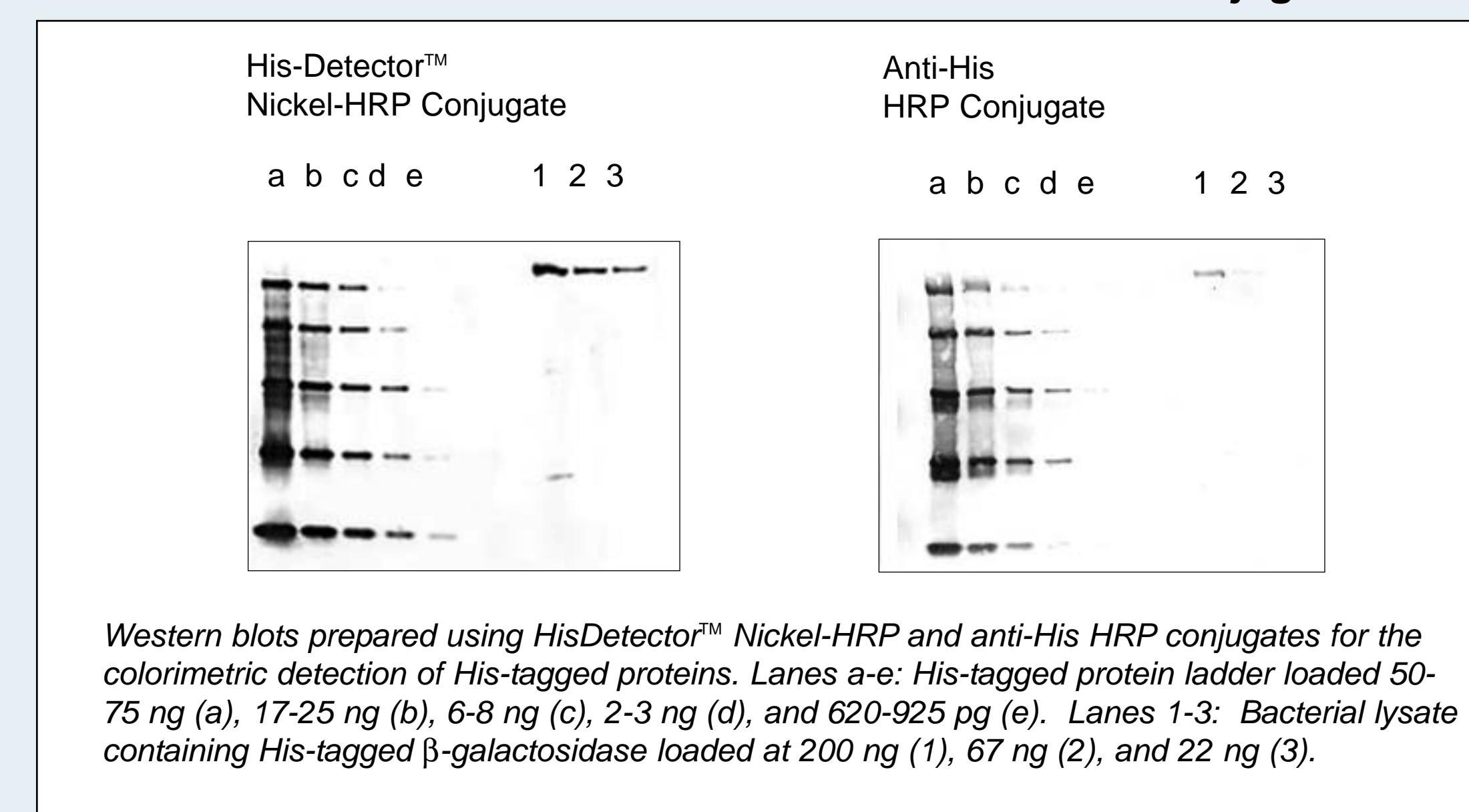
## METHODS

All materials, unless otherwise specified, were from KPL. Other supplies were obtained as follows: SDS-PAGE gel and Western blotting reagents/supplies (Bio-Rad Laboratories); anti-His HRP conjugate and the His-tagged protein ladder (Qiagen Inc.); Hek and Sf9 lysates (Kemp Biotechnologies, Inc., Frederick, MD). *E. coli* strain BL21-DE3 pLysS containing overexpressed His-tagged  $\beta$ -galactosidase was obtained from Novagen, Inc and the protein was purified by immobilized metal affinity chromatography (Qiagen) Western blots with HisDetector™ Ni-HRP or Ni-AP and with the anti-His HRP conjugate were performed according to the manufacturers' instructions. For colorimetric detection, TMB and BCIP/NBT were used to detect HRP and AP, respectively. LumiGLO® and PhosphaGLO™ were used similarly for chemiluminescent detection.

## RESULTS

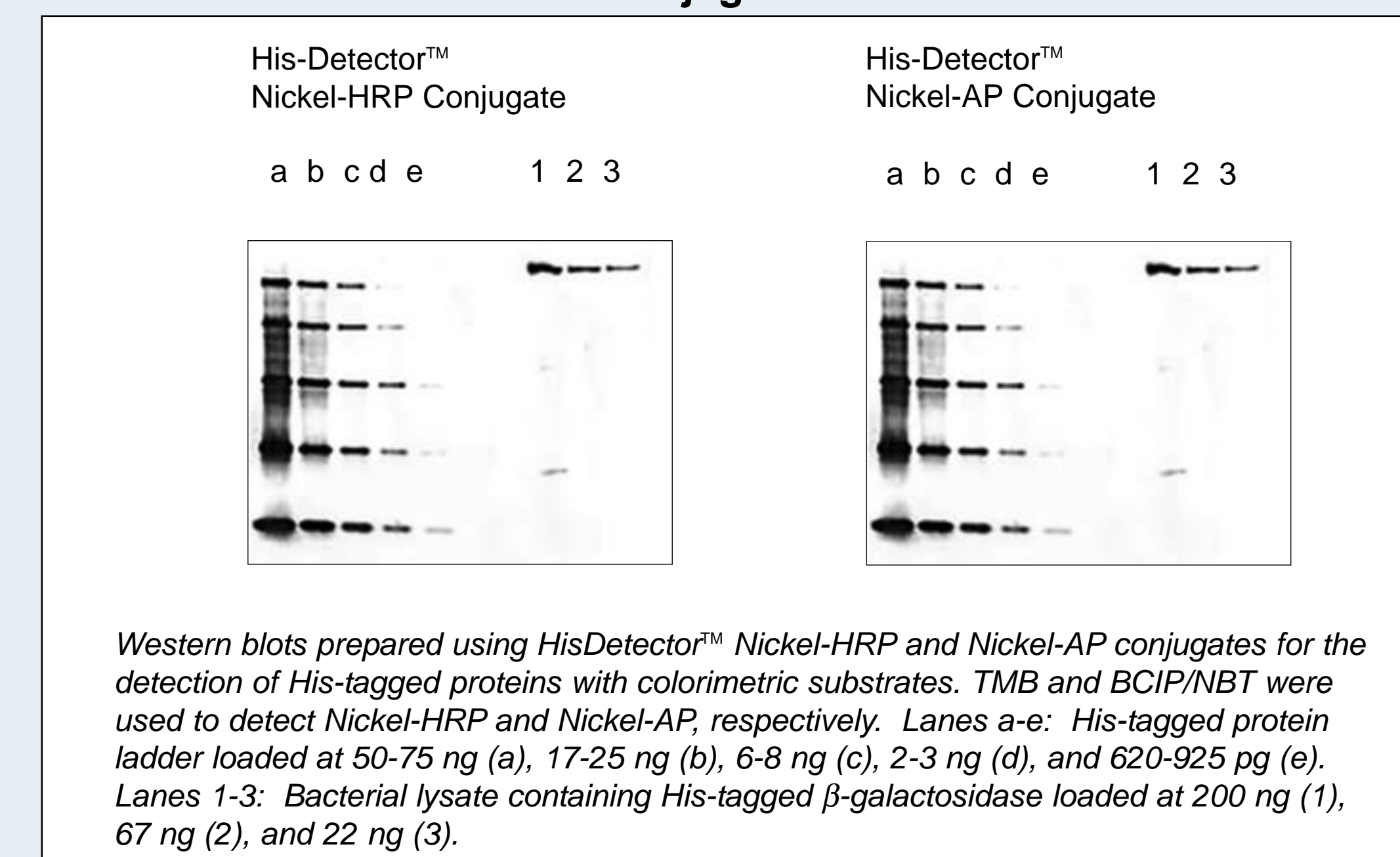
- Figure 3: Picogram levels of His-tagged protein were detected on Western blots with KPL's HisDetector™ Nickel-HRP conjugate.
- Lower levels of tagged protein were detected using HisDetector™ Nickel-HRP as opposed to using a commercially available anti-His antibody-enzyme conjugate.
- Similar results were observed with chemiluminescent detection (not shown).

Figure 3: Detection of His-tagged proteins: Performance Comparison of HisDetector™ Ni-NTA-HRP and anti-His-HRP Conjugates



- Figure 4: Picogram levels of His-tagged proteins were effectively detected on Western blots with either HisDetector™ Nickel-HRP or HisDetector™ Nickel-AP.
- Similar results were observed with chemiluminescent detection (not shown).

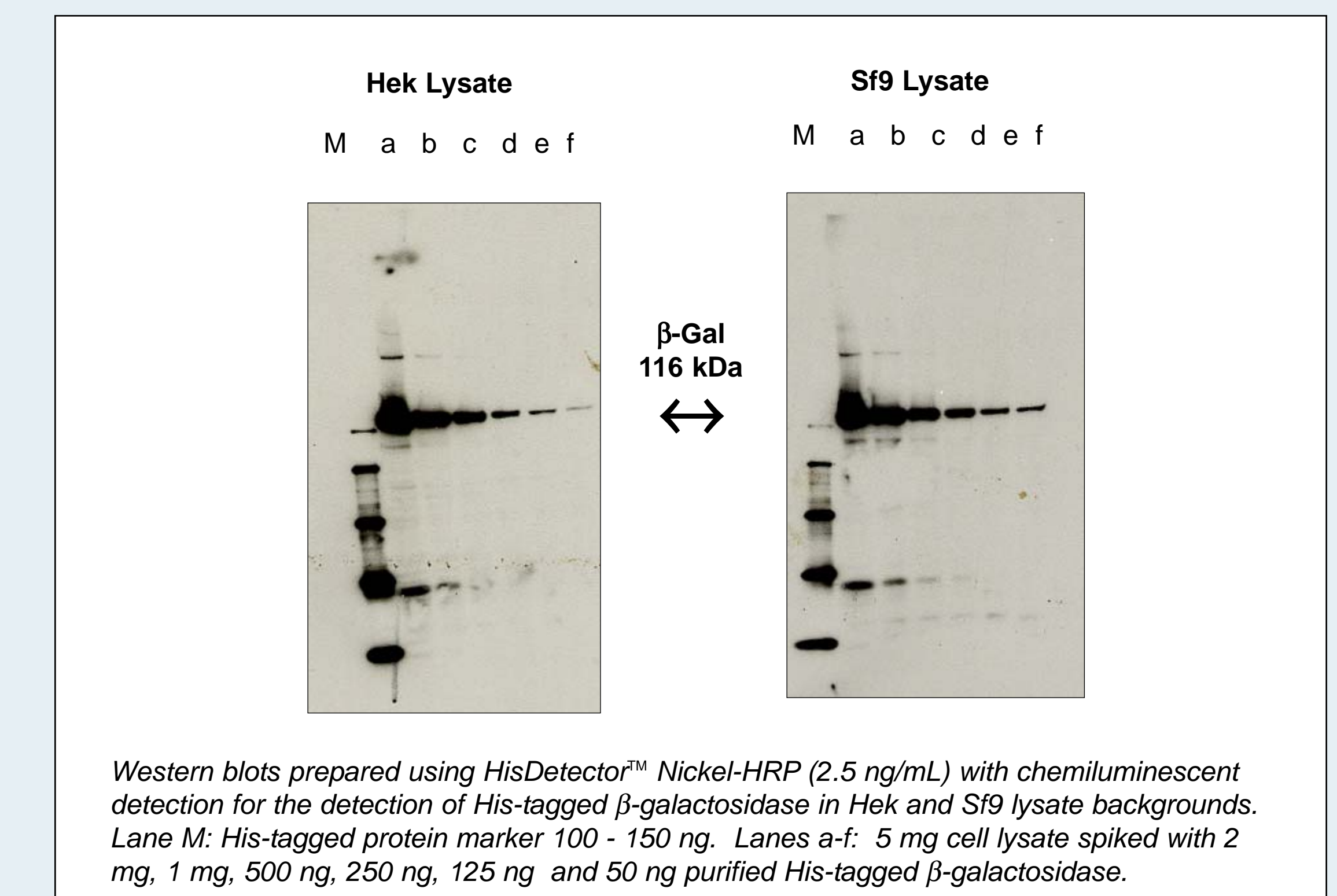
Figure 4: Detection of His-tagged Proteins Using HisDetector™ Ni-HRP and Ni-AP Conjugates



## RESULTS (Continued)

- Figure 5: His-tagged  $\beta$ -galactosidase was detected in human and insect cell lysates with HisDetector™ Nickel-HRP even when the His-tagged protein contributed <1% of the cell lysate protein mass.

Figure 5: Detection of His-tagged Proteins in Cell Lysates with HisDetector™ Ni-HRP



- Table 1: The total time and number of steps required for each detection method were determined.
- The antibody method was the most complex, requiring 8 steps.
- KPL's method was the most rapid, even compared to an alternate supplier of nickel-NTA-enzyme conjugates.

Table 1. Comparison of the Speed and Simplicity of Different Methods for Detecting His-tagged Proteins

Supplier	Technology	Detection Time (hrs)	Number of Steps
KPL	HisDetector Nickel-NTA	2.5	4
Supplier A	(Alternative) Nickel-NTA	3.25	5
Supplier B	Cobalt-Biotin/ Streptavidin	3.75	7
Supplier C	Antibody-HRP Conjugate	3.5	8

## CONCLUSIONS

- HisDetector™ Nickel-HRP and Nickel-AP can detect picogram levels of His-tagged proteins in cell extracts with low background in both colorimetric and chemiluminescent Western blot assays.
- The sensitivity of this method is superior to that of the antibody-based methods.
- The HisDetector™ protocol is the most rapid Western blot protocol available for detecting His-tagged protein.