LGC CLINICAL DIAGNOSTICS

Enzyme-Linked ImmunoSorbent Assay

See more with KPL ELISA products — Get higher signal and lower background.

seracare

See more with KPL ELISA Products

Sometimes it is difficult to distinguish your protein of interest from background. However, with **KPL Protein Detector™** ELISA products you get strong, clean signal every time. KPL's ELISA products offer consistent, high quality results and enable sensitive immunodetection of protein in a microwell format.

Choose from a broad line of sensitive substrates, high quality kits and stand-alone reagents for detecting and quantifying specific antigens in a complex protein mixture. Combine these products with KPL's extensive line of secondary antibodies to develop reproducible and sensitive ELISAs.

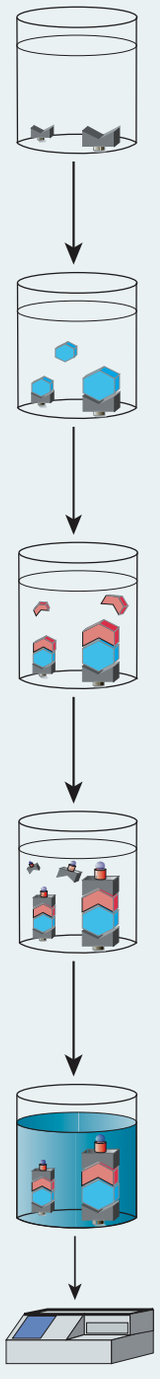
- **Substrates** for colorimetric or chemiluminescent detection
- **Secondary antibodies** unlabeled or labeled with either horseradish peroxidase (HRP) or alkaline phosphatase (AP)
- **Support reagents** – blocking solutions, coating buffers, wash solutions, conjugate stabilizers, stop solutions
- **Universal ELISA** kits formatted with HRP- or AP-labeled secondary antibodies

The foundation of KPL's Protein Detector ELISA offering is a broad line of chemiluminescent and chromogenic substrates for use with HRP and AP conjugates that provide varied levels of sensitivity. For highly sensitive colorimetric peroxidase detection, choose SureBlue Reserve™ Microwell Substrate with long shelf life and convenient 1-component format. New FirePhos™ Microwell Substrate expands our selection of sensitive substrates for phosphatase detection. LumiGLO Reserve™ Chemiluminescent Substrate offers the ultimate in sensitivity via chemiluminescent reaction with HRP. No matter which you choose, KPL's ELISA substrates are all tested to assure lot-to-lot consistency, reproducibility and long-term stability.

An extensive line of high-quality secondary antibody conjugates and labeled streptavidin are also available. Each is affinity purified to assure excellent signal-to-noise results in ELISAs. KPL support reagents are optimized for use in microwell ELISA and tested to verify that they meet strict specifications. They include conjugate stabilizers, diluents, blocking and wash solutions, all formulated to ensure accurate and reproducible results.

Protein Detector Kits provide a convenient starting point for the development of ELISA protocols. Each kit contains enzyme-labeled secondary antibody, corresponding substrate, as well as unique diluent/blocking and wash solutions.

Improving Signal-to-Noise in Protein Detection ELISA



1. **Immobilize primary antibody.**
   Coating solutions provide optimal buffers for binding antigen or antibody to solid phase.
2. **Block plate.**
   Blocking minimizes non-specific binding to solid phase and is critical for lowering background.
3. **Add protein mixture.**
4. **Wash.**
   Mild detergent removes unbound of low binding proteins. Soaking can improve wash effectiveness.
5. **Add primary antibody to specific protein.**
   Dilution of primary antibody in diluent/ blocking solution used to block plate minimizes non-specific binding to plate.
6. **Wash.**
7. **Add enzyme-labeled secondary antibody.**
   Specificity and sensitivity of antibody conjugate are critical to assay performance. Stabilizers maintain activity of conjugates.
8. **Wash.**
9. **Add enzyme substrate.**
   Substrate sensitivity, background, and stability are essential to ELISA performance.
10. **Stop substrate reaction and read.**
    Stopped substrate reactions provide consistent endpoint analysis and flexibility as to when assay results are recorded.

KPL ELISA products help you to optimize your assay and SEE MORE!

Praying Mantis

See more with KPL ELISA products — Get higher signal and lower background.

ELISA Substrates

Substrates are the cornerstones for developing ELISAs with high signal and low background. KPL, one of the first companies to produce stable, liquid substrates, offers a variety of standard and proprietary HRP and AP substrates. See Table 1 for a listing of chromogenic and chemiluminescent substrates.

Chromogenic Peroxidase Substrates

Choose from among three TMB (3,3',5,5'-tetramethylbenzidine) products and two ABTS (2,2"-azino-di-(3-ethylbenzthiazoline -6-sulfonate) formulations. All provide guaranteed performance and reliability.

Excellent Signal-to-Noise with SureBlue™

- **High sensitivity**
- **Consistent lot-to-lot performance minimizes assay re-optimization**
- **2-year stability**

KPL's SureBlue TMB Microwell Substrate is a 1-component formulation. It develops a deep blue soluble product when reacted with HRP-labeled conjugates. SureBlue TMB provides excellent signal-to-noise, stability, and consistency, making it the ideal substrate for use in most ELISA applications.

Superior Sensitivity with SureBlue Reserve

- **Highest sensitivity: 50% more sensitive than SureBlue TMB (Figure 1)**
- **Consistent lot-to-lot performance minimizes assay re-optimization**
- **2-year stability**
- **Ready-to-use 1-component formulation**

SureBlue Reserve TMB takes ELISA to a new performance level. It offers maximum sensitivity with exceptionally low background enabling detection of low levels of target protein. SureBlue Reserve TMB provides the same great benefits as KPL's original SureBlue TMB: low lot-to-lot variation, 2-year stability, ready-to-use 1-component solution, and excellent linearity.

TMB Microwell Substrate in a Highly Stable 2-Component Formulation

- Longest shelf life due to the 2-component system
- Higher sensitivity than ABTS

TMB Microwell 2-Component Substrate offers excellent stability and sensitivity. It is the most economical choice when ready-to-use convenience is not required. Like other TMB substrates, it rapidly develops a deep blue color that is measurable at 650 nm.

Broad Dynamic Range with ABTS Microwell Substrates

- **Excellent linearity; provides the widest working range of any chromogenic HRP substrate**
- **Extremely stable**
- **Ideal for kinetic assays**

KPL's ABTS Microwell Substrates offer performance, low background, and a wider linear range than other chromogenic substrates. Supplied either as a ready-to-use formulation or as an extremely stable 2-component product, ABTS substrates develop an intense blue-green color in the presence of peroxidase-labeled conjugates. ABTS is recommended when low background is required and moderate sensitivity is acceptable.

Table 1. KPL Protein Detector ELISA Substrates

	SENSITIVITY	STABILITY	CONVENIENCE	ENZYME	DETECTION	DETERMINATION
SureBlue Reserve	●●●	●●●	●●●●●	HRP	Chromo	Kinetic or Endpoint
SureBlue	●●	●●●	●●●●●	HRP	Chromo	Kinetic or Endpoint
TMB 2-C	●●	●●●●	●●●	HRP	Chromo	Kinetic or Endpoint
ABTS 1-C	●	●●●●	●●●●●	HRP	Chromo	Kinetic or Endpoint
ABTS 2-C	●	●●●●●	●●●	HRP	Chromo	Kinetic or Endpoint
BluePhos	●●	●●	●●●	AP	Chromo	Kinetic or Endpoint
LumiGLO Reserve	●●●●●	●●	●●●	HRP	Chemi	Endpoint
LumiGLO	●●●●	●●	●●●	HRP	Chemi	Endpoint
PhosphaGLO	●●●●	●●●	●●●●●	AP	Chemi	Kinetic or Endpoint
PhosphaGLO Reserve	●●●●●	●●●	●●●●●	AP	Chemi	Kinetic or Endpoint

C = Component ● = Lowest ●●●●● = Highest

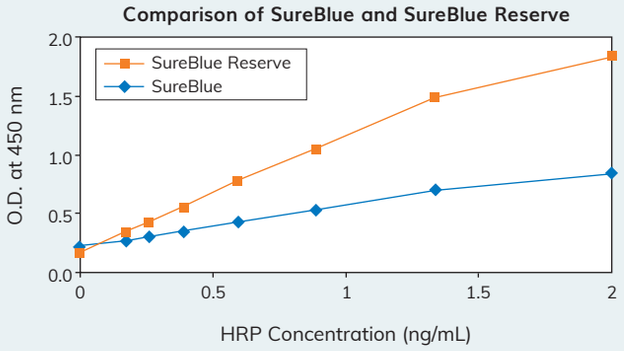


Figure 1. Dilutions of HRP conjugate were added to microplate wells and the conjugate in each well reacted with either SureBlue or SureBlue Reserve. The reactions were stopped with TMB Stop Solution, and optical density readings were taken at 450 nm. Data points represent the average of triplicate samples at each HRP concentration. The data demonstrate greater sensitivity of SureBlue Reserve at low concentrations of HRP.

KPL BluePhos - Chromogenic Phosphatase Substrate

For economical chromogenic phosphatase detection that provides sensitivity and convenience, KPL offers BluePhos, a chromogenic substrate with an exceptional linear range.

Excellent Overall Performance with BluePhos®

- **Ideal for applications where sensitivity greater than pNPP is required**
- **Stable for 24 months**
- **Convenient and quick: mix and use**
- **Wider dynamic range than pNPP**
- **Lower background than pNPP**

BluePhos Microwell Substrate is based on a soluble, proprietary form of 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and produces intense color. This substrate offers greater sensitivity and linearity of signal than pNPP (Figure 2) and can be used for both kinetic and endpoint ELISAs.

Chemiluminescent Substrates for Peroxidase and Phosphatase

LumiGLO® and LumiGLO Reserve Chemiluminescent Peroxidase Substrates

- **Unsurpassed sensitivity with LumiGLO Reserve**
- **Excellent stability**
- **Fast ramp-up of signal**
- **Stable for 24 hours after mixing**
- **Light emission sustained for 1-2 hours**

LumiGLO™ and LumiGLO Reserve™ substrates are luminol-based. In the presence of hydrogen peroxide, they are converted to an excited intermediate dianion which emits light on return to its ground state.

Both LumiGLO Reserve™ and LumiGLO™ substrates offer rapid sensitive detection. The working range of primary antibody that yields an acceptable signal-to-noise ratio is much wider with these substrates than with chromogenic substrates. Both substrates are supplied as 2-component liquids.

PhosphaGLO™ and PhosphaGLO Reserve™ Chemiluminescent Phosphatase Substrates

- **Femtogram sensitivity with PhosphaGLO Reserve**
- **One-component, ready-to-use**
- **Stable at 4° C for two years**

PhosphaGLO Reserve™ and PhosphaGLO™ substrates provide great sensitivity and unmatched convenience. No special blockers are required to achieve low background.

ELISA Antibody Conjugates

Choose from a wide selection of secondary antibodies to immunoglobulins from over 20 animal species labeled with horseradish peroxidase or alkaline phosphatase, the most widely used enzymes for ELISA signal generation. HRP has been shown to be more sensitive than AP primarily due to its faster catalytic rate. However, HRP reactions may be self-limiting due to substrate inhibition of enzyme. In contrast, AP exhibits a slower catalytic rate, but is not self-limiting. Reaction rates remain linear over long periods of time; therefore, sensitivity can be improved by allowing the reaction to proceed for a longer time.

Whatever enzyme conjugate you choose, all antibody conjugates are of the same exceptionally high quality you have come to expect from KPL. As always, you get results with high signal-to-noise.

Universal ELISA Kits

KPL's Protein Detector ELISA Kits are designed to be an excellent and convenient starting point for development of ELISA protocols. Each kit contains all the reagents necessary to establish an ELISA procedure except for primary antibodies. The kits also include a comprehensive manual to facilitate assay development.

HRP ELISA Kits

KPL's Protein Detector HRP ELISA Kits contain goat anti-mouse IgG and anti-rabbit IgG, or anti-human IgG HRP conjugates along with ABTS substrate, blocking, coating, substrate, stop and wash solutions.

AP ELISA Kits

Included in the Protein Detector AP ELISA kits are goat anti-mouse IgG, anti-rabbit IgG, or anti-human IgG AP conjugates as well as BluePhos substrate, blocking, coating, stop and wash solutions.

ELISA Support Reagents

KPL's support reagents are optimized for use in microwell ELISA and tested to verify that they meet strict specifications. They include conjugate stabilizers, diluents, blocking and wash solutions, and stop reagents. Blocking and wash solutions have been designed to reduce background and to ensure a clear signal. KPL stabilizers provide convenient storage solutions for your antibody-enzyme conjugates and ensure their performance.

Spend your time developing your assay instead of worrying about making reagents. Choose time-tested, stable support reagents from KPL.

SEE MORE...

Try KPL's ELISA Kits and Reagents

KPL has extensive experience in antibody purification and the development of stable liquid substrates. This long-standing expertise is the foundation of our line of Protein Detector ELISA systems and ensures reliable product performance from lot-to-lot.

Try our kits and reagents and get the results you're looking for: a strong, clean signal without background, every time.

Your eyes aren't playing tricks on you. You've found the finest line of ELISA systems and reagents at KPL.

Ordering Information

Protein Detector ELISA Kits

Each kit includes an AP- or HRP-labeled Conjugate, Coating Solution, Wash Solution Concentrate, 10% BSA Diluent/ Blocking Solution Concentrate, Stop Solution and Substrate.

MATERIAL #	PRODUCT DESCRIPTION	SIZE
PHOSPHATASE		
5110-0012 (55-81-10)	AP ELISA Kit, Anti-Human	20 plates
PEROXIDASE		
5110-0009 (54-62-10)	HRP ELISA Kit, Anti-Human	20 plates
5110-0011 (54-62-18)	HRP ELISA Kit, Anti-Mouse	20 plates
5110-0010 (54-62-15)	HRP ELISA Kit, Anti-Rabbit	20 plates

Substrates for ELISA

PHOSPHATASE CHROMOGENIC SUBSTRATES		
5120-0061 (50-88-02)	BluePhos Microwell Substrate Kit	50 mL
5120-0059 (50-88-00)	Same as above	600 mL
5120-0060 (50-88-01)	Same as above	2.7 L
PEROXIDASE CHROMOGENIC SUBSTRATES		
5120-0075 (52-00-01)	SureBlue TMB 1-Component Substrate	100 mL
5120-0076 (52-00-02)	Same as above	400 mL
5120-0077 (52-00-03)	Same as above	1 L
5120-0078 (52-00-04)	Same as above	5 L
5120-0081 (53-00-01)	SureBlue Reserve TMB 1-Component Substrate	100 mL
5120-0082 (53-00-02)	Same as above	400 mL
5120-0083 (53-00-03)	Same as above	1 L
5120-0047 (50-76-00)	TMB 2-Component Substrate Kit	600 mL
5120-0050 (50-76-03)	Same as above	2.7 L
5120-0046 (50-66-18)	ABTS Microwell Substrate 1-Component	100 mL
5120-0041 (50-66-00)	Same as above	600 mL
5120-0043 (50-66-06)	Same as above	1 L
5120-0032 (50-62-00)	ABTS Substrate System 2-Component	600 mL
5120-0033 (50-62-01)	Same as above	2700 mL
PEROXIDASE CHEMILUMINESCENT SUBSTRATES		
5430-0040 (54-61-00)	LumiGLO Chemiluminescent Substrate Kit	240 mL
5430-0041 (54-61-01)	Same as above	720 mL
5430-0042 (54-61-02)	Same as above	60 mL
5430-0049 (54-71-00)	LumiGLO Reserve Chemiluminescent Substrate Kit	2400 cm^2
5430-0050 (54-71-01)	Same as above	600 cm^2
5430-0051 (54-71-02)	Same as above	50mL
PHOSPHATASE CHEMILUMINESCENT SUBSTRATES		
5430-0054 (55-60-03)	PhosphaGLO AP Substrate	30 mL
5430-0055 (55-60-04)	Same as above	100 mL
5430-0052 (55-60-01)	PhosphaGLO Reserve AP Substrate	30 mL
5430-0053 (55-60-02)	Same as above	100 mL

Antibody Conjugates

All antibodies listed below are produced in goat. For a complete antibody listing, visit www.kpl.com or refer to KPL's Product Catalog.

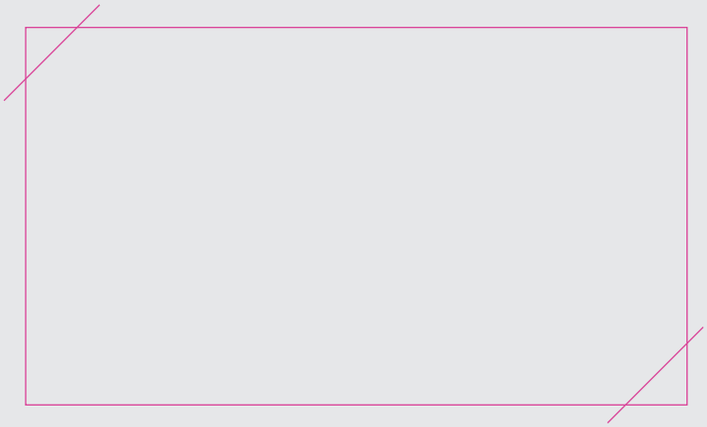
MATERIAL #	PRODUCT DESCRIPTION	SIZE
PHOSPHATASE		
5220-0351 (0751-1006)	ReserveAP Anti-Human IgG (H+L)	1.0 mg
5220-0357 (0751-1806)	ReserveAP Anti-Mouse IgG (H+L), HSA	1.0 mg
5220-0353 (0751-1506)	ReserveAP Anti-Rabbit IgG (H+L)	1.0 mg
PEROXIDASE		
5220-0330 (074-1006)	Anti-Human IgG (H+L)	1.0 mg
5220-0341 (074-1806)	Anti-Mouse IgG (H+L), HSA	1.0 mg
5220-0336 (074-1506)	Anti-Rabbit IgG (H+L)	1.0 mg
BIOTIN-LABELED		
5260-0031 (16-10-06)	Anti-Human IgG (H+L)	0.5 mg
5260-0048 (16-18-06)	Anti-Mouse IgG (H+L), HSA	0.5 mg
5260-0038 (16-15-06)	Anti-Rabbit IgG (H+L)	0.5 mg
LABELED STREPTAVIDIN		
5270-0030 (15-30-00)	AP-labeled	0.5 mg
5270-0029 (14-30-00)	HRP-labeled	0.5 mg

Assay Support Reagents

ASSAY SUPPORT REAGENTS		
5150-0014 (50-84-00)	Coating Solution Concentrate	50 mL
5150-0008 (50-63-00)	Wash Solution Concentrate	800 mL
5150-0008 (50-63-00)	Wash Solution Concentrate (20x)	1000 mL
BLOCKING SOLUTIONS		
5140-0006 (50-61-00)	10% BSA Diluent/ Blocking Solution	200 mL
5140-0008 (50-61-10)	10% BSA Diluent/ Blocking Solution	1 L
5140-0011 (50-82-01)	Milk Diluent/Blocking Solution	200 mL
CONJUGATE STABILIZERS		
5290-0007 (55-15-00)	APStabilizer	200 mL
5290-0005 (54-15-01)	HRPStabilizer	200 mL
STOP SOLUTIONS		
5150-0017 (50-85-01)	ABTS Stop Solution	200 mL
5150-0026 (50-89-00)	APstop Solution	200 mL
5150-0020 (50-85-05)	TMB Stop Solution	400 mL
5150-0021 (50-85-06)	TMB Stop Solution	1 L
5150-0022 (50-85-30)	TMB BlueSTOP Solution	400 mL
5150-0024 (50-85-41)	TMB BlueSTOP Solution	1 L

HSA: Human serum adsorbed

ISO 13485: 2016 Registered



Phone: 800.638.3167; 301.948.7755
Fax: 301.948.0169
kpl.com