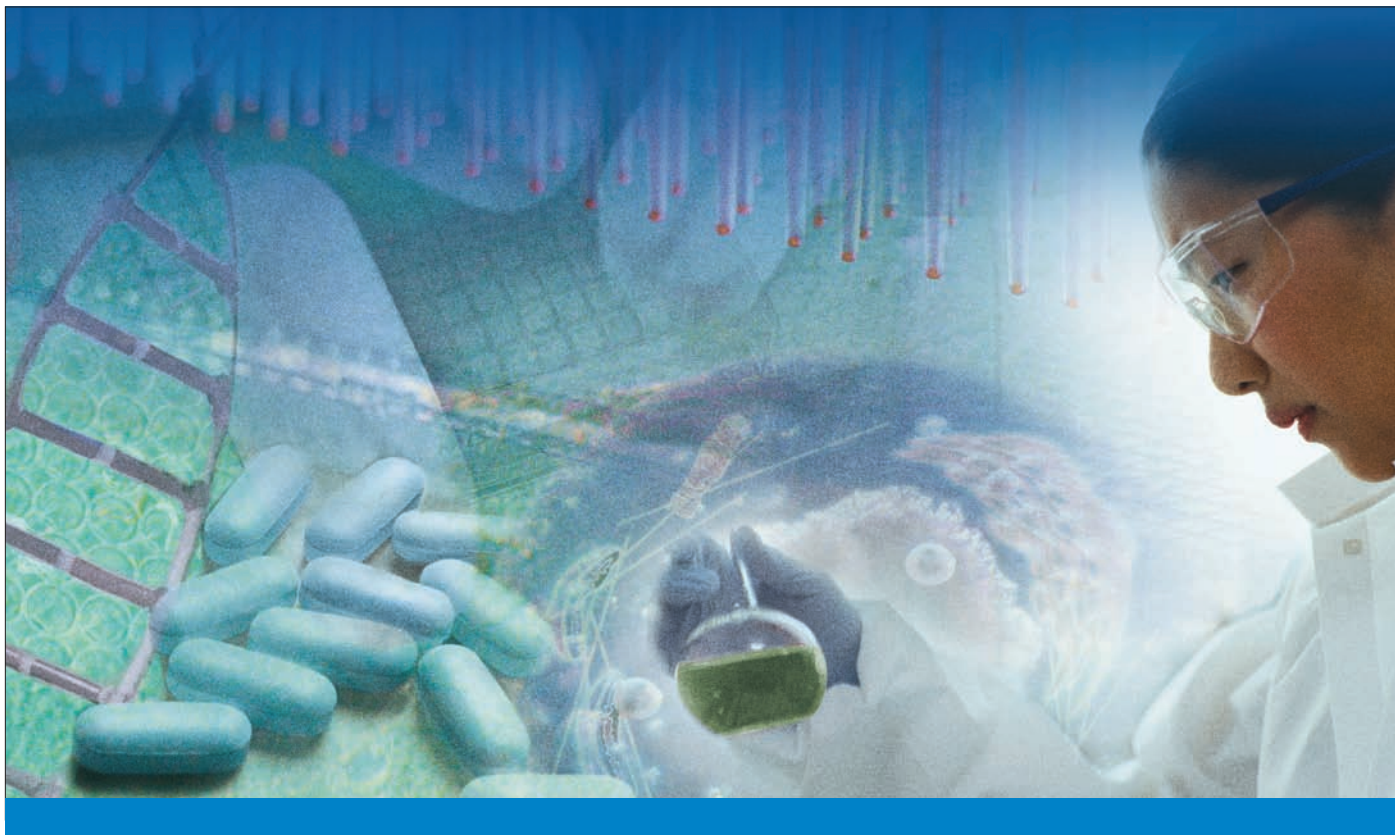


## ELAST ELISA

# Amplification System

for the **results** you need, **quickly!**



### Overview

Enzyme-linked immunosorbent assay (ELISA) is a sensitive immunoassay that uses an enzyme linked to an antibody or antigen as a marker for the detection of a specific protein, especially an antigen or antibody. ELISAs are utilized to detect or quantitate analytes in a sample. A significant diagnostic application of ELISAs is in the determination of exposure to an infectious agent such as the AIDS virus, by identifying antibodies present in a blood sample.

The PerkinElmer ELAST® (ELISA Amplification System) is designed to amplify the signal generated by the enzyme horseradish peroxidase (HRP) in solid phase analytical methods such as ELISA. ELAST is based on the use of PerkinElmer's proprietary Tyramide Signal Amplification (TSA) technology.

The ELISA reporter enzyme, HRP, catalyzes the covalent binding of biotinyl-tyramide to the solid phase of assay wells. Subsequent reaction with streptavidin-HRP causes additional HRP to be bound to the solid phase resulting in signal amplification.

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### Key Benefits

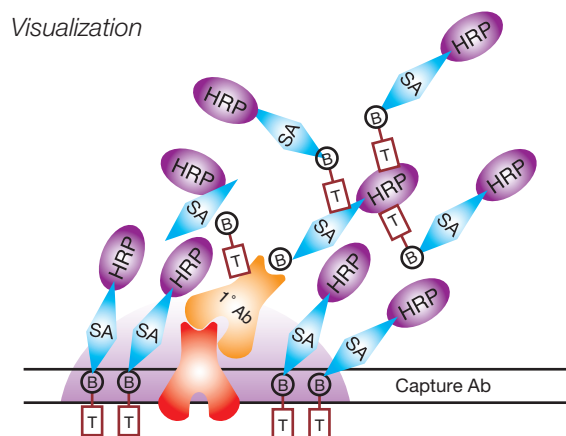
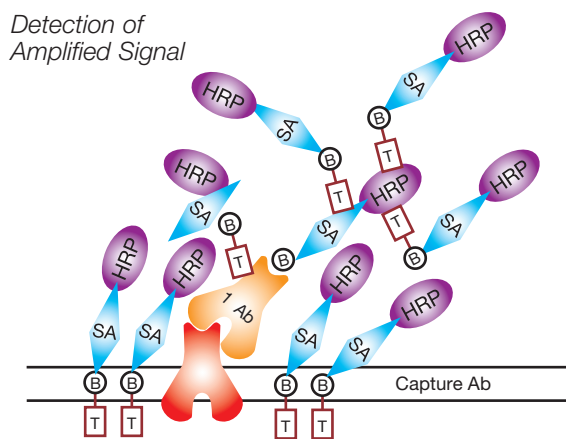
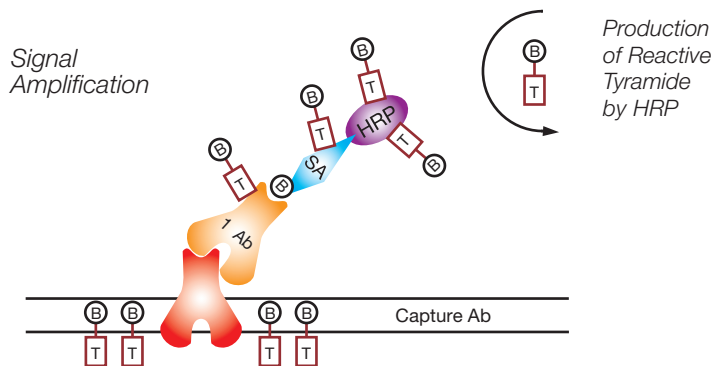
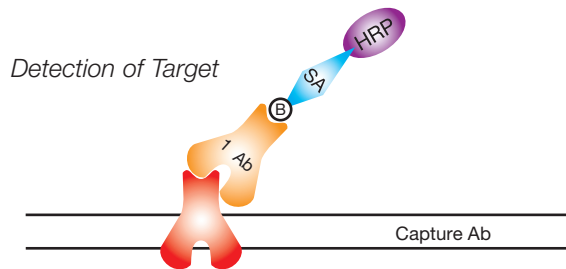
- **Ideal for detection** of low abundant analytes.
- **Compatible with a variety of different sample formats**, including serum and plasma.
- **Improves sensitivity** of *any* HRP-based ELISA 8- to 32-fold with equal sample size.
- **Consumes less of your precious samples** to obtain equal sensitivity.
- **Inserts directly into your protocol** – adds only two quick steps.
- **Includes reagents** for ten 96-well microliter plates.
- **Detection of a wide range of analyte sizes** from small hormones to bulky complexes.

**PerkinElmer**  
precisely.

# TSA ELAST Detection

(Biotin-based)

## ELISA



Amplification and Visualization Procedures	Actual Time Added to Protocol
Blocking Step	30 min
Wash Step	4 x 5 min
Incubation of biotinyl-tyramide	3–10 min
Wash Step	4 x 5 min
Addition of enzyme conjugate	30 min
Wash Step	4 x 5 min
Incubation of chromogen	

- Tyramide Signal Amplification is based on the patented catalyzed reporter deposition (CARD) technique using derivatized tyramide.
- Hydrogen peroxide and HRP convert the labeled substrate (tyramide) into a short-lived, extremely reactive intermediate.
- Activated substrate molecules then rapidly react with and covalently bind to electron rich regions of adjacent proteins.
- Binding of the activated tyramide molecules occurs only immediately adjacent to the sites at which the activating HRP enzyme is bound.
- Multiple deposition of the labeled tyramide occurs in a very short time (generally within 3-10 minutes).
- Subsequent detection of the label yields an effectively large amplification of signal.

# ELAST Kits

## ELAST ELISA Kit

PerkinElmer offers a complete package of reagents necessary to employ catalyzed reporter deposition, the NEN ELAST ELISA Kit. This amplification method is extremely flexible and easy to use. Except for the biotinylated-phenolic compounds, common immunoassay reagents are employed. Note that the EIA to be amplified must employ HRP as the reporter enzyme and that the appropriate blocking proteins should be used in preparation of the microplates (i.e. bovine serum albumin works quite well). Only three reagents are required and are supplied with this system:

- Biotinyl-Tyramide Solution
- Amplification Diluent Concentrate (2X)
- Streptavidin-HRP Concentrate

These three components are included in the ELAST System, along with an easy-to-follow instruction manual which contains a comprehensive example section showing the improvements in sensitivity obtained with some typical ELISA systems.

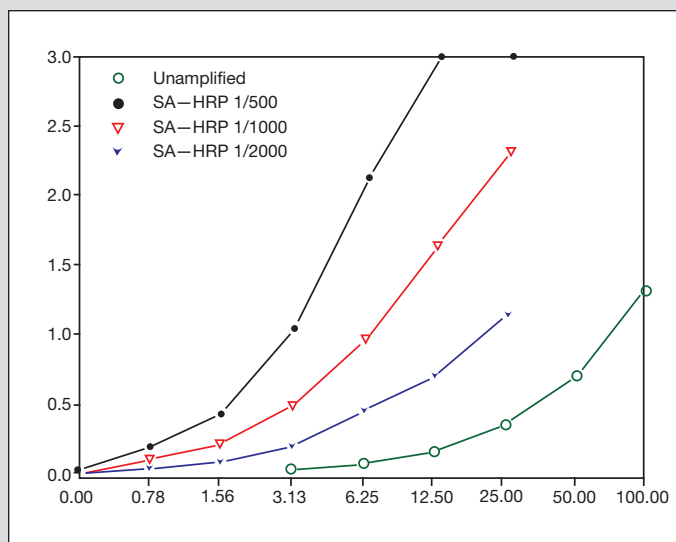
*These kits are intended for research purposes only and are not approved for diagnostic use.*

## Alliance HIV-1 p24 Antigen Ultra-sensitive ELISA

Combine our HIV-1 p24 Antigen ELISA Kit with our ELAST ELISA Amplification System for ultra-sensitivity. The assay is based on the conventional ELISA technique but with an added signal amplification step (ELAST). This provides an extended detection range from 1 to 6,500 pg, without loss of linearity. The extended range will also reduce the need to dilute your samples.

The assay is performed as usual through the addition of the streptavidin-HRP conjugate. After incubation of the conjugate, ELAST reagents are added and incubated for 30 minutes, enhancing the ortho-phenylenediamine-HCl (OPD) signal and the detection potency.

### Optimization of ELAST ELISA Kit



A titration of the ELAST streptavidin-HRP concentrate was made 1/500, 1/1000, and 1/2000 in 1% BSA-PBS-T. One dilution was used for each standard curve. 100  $\mu$ l was added to the wells and incubated for 15 minutes at room temperature. The wells were washed as recommended.

OPD substrate was added and incubated for 30 minutes at room temperature, then stopped with the kit stop solution. The absorbance at 490 nm was determined.

The 1/500 dilution yielded the highest absolute signal (over 20 times the unamplified assay) as well as net signal (background subtracted). On the other hand, the 1/1000 dilution yields greater than 10-fold signal increase with the highest signal-to-noise ratio due primarily to the lower background. Therefore, the best improvement in assay sensitivity is achieved with the 1/1000 dilution.

# Ordering Information

## ELAST ELISA Amplification System/Virology Kits & Reagents

Product	Description	Product No.
ELAST ELISA Amplification System	Contains reagents to amplify samples in ten 96-well ELISA microplates.	NEP116001EA
	For use with the Alliance HIV-1 p24 Antigen ELISA Kit	NEP116VL001PK

## Virology Kits & Reagents

Product	Description	Product No.
Alliance HIV-1 p24 Antigen ELISA Kit	One 96-well plate and reagents for 96 tests	NEK050001KT
	Two 96-well plates and reagents for 192 tests	NEK050A001KT
	Five 96-well plates and reagents for 480 tests	NEK050B001KT
Alliance HIV-1 p24 Antigen Ultra-sensitive ELISA Kit	Five 96-well plates and reagents for 480 tests	NEK050B001KT and NEP116VL001PK
Alliance HIV-1 p24 Antigen Confirmatory Reagent	100 tests	NEK059001EA
ELISA Plate Wash	3 L	NEA107001EA
ELAST ELISA Amplification System	HIV viral antigen detection amplification system	NEP116VL001PK

## Alliance Monoclonal Antibodies and HIV Research Reagents

Product	Description	Product No.
<b>Monoclonal Antibodies</b>		
CMV monoclonal antibody, (Early Nuclear Protein)	100 µg IgG <sub>2A</sub>	NEA9221100UG
HIV gp120 monoclonal antibody, (sequence specific, mouse)	0.5 mL	NEA9301001EA
HIV gp120 neutralizing monoclonal antibody, purified, (sequence specific)	0.5 mL	NEA9205001EA
HIV gp41 monoclonal antibody, (mouse)	0.5 mL	NEA9303001EA
HIV p24 Site 1A monoclonal antibody, (mouse)	0.5 mL	NEA9306001EA
HIV reverse transcriptase monoclonal antibody, (mouse)	0.5 mL	NEA9304001EA
<b>Reagents</b>		
3'-Azido-3'-Deoxythymidine-5'-Triphosphate (AZT-TP)	10 mM/250 µL	NEI491001EA
CD4, recombinant, soluble	100 µg	NEA200001EA
HIV p24 core peptide, (recombinant)	100 µg/0.5 mL	NEA210001EA
HIV gp41 envelope peptide, (recombinant)	100 µg/0.5 mL	NEA211001EA
HIV gp120, (recombinant)	Made to order	NEA201001EA

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