

## PolyHRP Users Guide

### Introduction to PolyHRP

- PolyHRP is an enhanced enzymatic label comprising covalent horseradish peroxidase polymer. PolyHRP conjugates deliver a large number of signal-generating enzyme molecules to one bound analyte molecule. This results in multiple detection enhancements which are directly proportional to the HRP polymerization range resulting in easy detection of low-abundance targets (low picogram-femtogram range) in immunoassays.

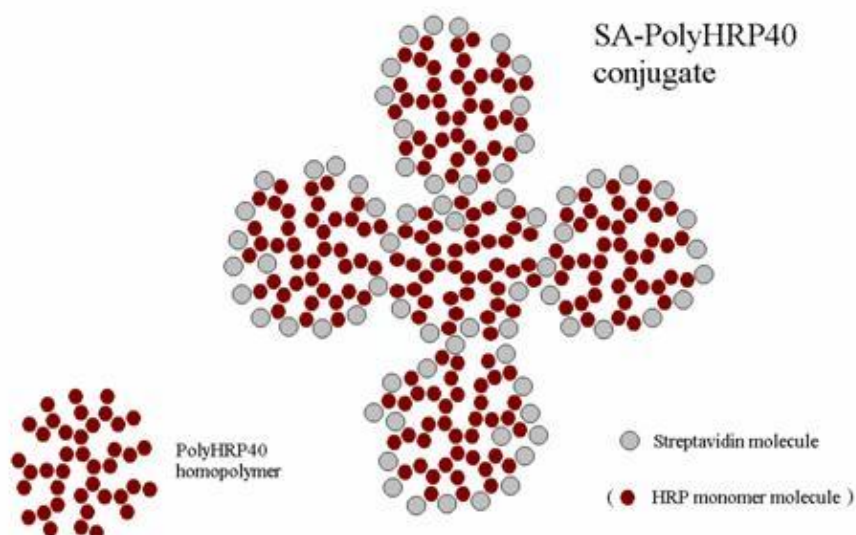


Figure 1. Simplified (2D) schematic representation showing molecular design of SA-PolyHRP40 conjugate

- Streptavidin-PolyHRP conjugates are made of five identical HRP homopolymer blocks that are covalently coupled to multiple streptavidin molecules. Three different homopolymers are currently used in the production process. These are PolyHRP20, PolyHRP40 and PolyHRP80. Streptavidin-PolyHRP80 is more sensitive than Streptavidin-PolyHRP40, and Streptavidin-PolyHRP40 is more sensitive than Streptavidin-PolyHRP20.

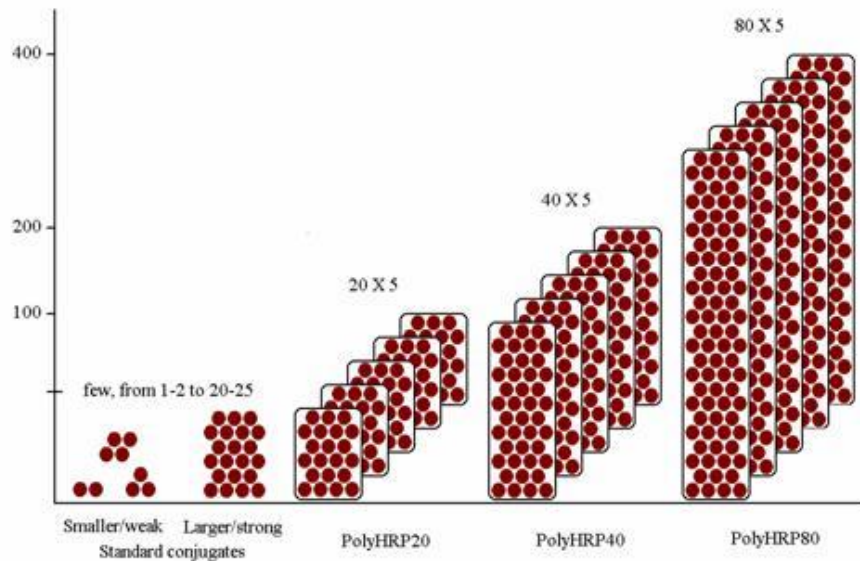


Figure 2. Number of HRP monomer molecules in different standard and PolyHRP conjugates

- Technically detection with Streptavidin-PolyHRP is simple. There are no major changes required that would affect an existing assay system (number of steps, incubation intervals etc). Required kit reagents are essentially the same as in conventional ELISA using standard Streptavidin-HRP.
- Similarly PolyHRP detection may be used with almost any HRP (colorimetric, fluorimetric) substrate development systems including enhanced chemiluminescence. This also means perfect compatibility with existent and emerging ELISA and immunohistochemistry instrumentation.
- Other analytical methods where PolyHRP detection can be advantageous include highly sensitive DNA/RNA-hybridization assays and ligand-receptor assays.

### Advantages of PolyHRP

- **Highly sensitive** - PolyHRP can detect extremely low-level targets (low picogram-femtogram range) with high signal-to-noise ratios
- **Flexible** - Compatible with chromogenic, fluorogenic and chemiluminescent substrates
- **Robust** - Reagent is highly pure and free of unconjugated molecules for highest specific activity and minimal background noise
- **Stable** - Ready-to-use liquid reagents can be stored at 4 deg C
- **Cost effective** - Conjugate requirements per assay are far less than conventional assays
- **Versatile** - Compatible with ELISA, Western blotting, immunohistochemistry and more
- **Easy to use** - Can be directly substituted into immunoassays and other detection assays

## **Typical procedure for Developing an Ultra-sensitive Antibody Sandwich ELISA Test System using Streptavidin-PolyHRP**

1. Use only highly pure deionised water and research grade reagents. Filter all working buffers through 0.1-0.2  $\mu\text{m}$  (semi) sterile filter devices, avoid foaming during the filtration process as excessive  $\text{O}_2$  content can damage HRP.
2. Use only High Binding Capacity Immunoplates; do not use low-medium capacity plates. When possible, use chromatographically pure specific IgG or antigen binding antibody fragments.
3. Coat immunoplates with capture antibody at 1  $\mu\text{g}/\text{ml}$  concentration. When using antibody fragments, establish optimum concentration in separate experiments. Use standard 50 mM Sodium Carbonate/Bicarbonate Buffer, pH 9.5, with 0.03% BND, 0.2  $\mu\text{m}$ -filtered.
4. Incubate immunoplates filled with the antibody solution for 18 hours at +4 deg C, sealed or placed into a clean, humid chamber.
5. Wash antibody-coated immunoplate with PBS containing 0.05% Tween-20 (PBS-Tween) 5 times. Allow plate to dry or optionally dry after additional single wash step with appropriate drying buffer containing dry reagent stabilizers, usually high concentration oligo/poly-sugars (trehalose, dextrans, dextrans) or natural (BSA, casein) or synthetic (PVA, PVP, etc.) supporting polymers.
6. Only dilute Streptavidin-PolyHRP conjugates with buffer systems that are biotin-free and contain an effective blocker of non-specific protein-protein binding. Universal (SA-PolyHRP) Casein Diluent/Blocker (catalog # 85R-111) and Streptavidin-PolyHRP Conjugate Stabilizers (catalog # 85R-112) are highly recommended for diluting SA-PolyHRP to final working strength.

If necessary users should be prepared to dilute the concentration of the biotinylated antibody being used in the detection system compared to concentration used in an existing ELISA test with standard Streptavidin-HRP.

7. Run the Chess titration experiment to generate a calibration curve for use in the final immunoassay, points on the curve should include
  - (i) Streptavidin-PolyHRP only, zero antigen, zero antibody-biotin.
  - (ii) Antibody-biotin and Streptavidin-PolyHRP only, zero antigen
  - (iii) Low antigen concentration
  - (iv) Medium antigen concentration
  - (v) High antigen concentration
8. Samples are measured at 450 nm vs. 620 nm.

**Notes on procedure:**

- SA-PolyHRP conjugates are very sensitive to minimal biotin content potentially present in diluents which users may currently utilise. Endogenous (both free and bound) biotin will naturally weaken detecting activity of any SA-HRP conjugate.
- Diluents made of animal sera as a rule are not compatible with Streptavidin-PolyHRP. Exhaustive dialysis and other methods do not completely remove bound biotin associated with medium and high molecular weight material and can still strongly inhibit Streptavidin-PolyHRP conjugates.
- Effective ultrasensitive performance with Streptavidin-PolyHRP generally requires application of detector antibody-biotin conjugate in smaller concentrations compared to that which would typically be used with standard Streptavidin-HRP.

## PolyHRP Products available from Fitzgerald

\* Prices applied on 01 May 12 (subject to change, check website for up to date pricing)

Catalog No.	Product Name	Size	Price
85R-113	Antibody/Antigen Conjugate Stabilizer (Poly-HRP)	100 ml	\$ 104.50
85R-111	Antibody/Antigen Conjugate Diluent/Blocker	100 ml	\$ 104.50
30R-AA034	Aminoethyl Poly-HRP40	5 mg	\$ 235.00
65R-P102	Protein A/G-Poly-HRP40	1 mg	\$ 624.75
65R-P100	Protein A-Poly-HRP20	1 mg	\$ 595.00
65R-P103	Protein A-Poly-HRP20	50 ug	\$ 250.00
65R-P101	Protein A-Poly-HRP40	1 mg	\$ 595.00
65R-P104	Protein A-Poly-HRP40	50 ug	\$ 250.00
55R-APBLOT	Protein A-Poly-HRP Blotting Developer Kit	1 kit	\$ 895.00
85R-124	Binding/Coating Buffer (10X)	100 ml	\$ 104.50
99R-104	5-Bromo-5-nitro-1,3-dioxane (powder)	5 grams	\$ 104.50
99R-105	5-Bromo-5-nitro-1,3-dioxane (solution)	100 ml	\$ 104.50
30R-AB022	Fraction V BSA 10% solution	10 ml	\$ 104.50
85R-107	Casein Buffer (20X-4X)	100 ml	\$ 434.50
85R-115	Stabilizing Buffer (BSA based)	100 ml	\$ 104.50
85R-116	Stabilizing Buffer (Casein based)	100 ml	\$ 104.50
85R-121	TMB Substrate	30 ml	\$ 104.50
43R-IG101PH	Goat anti Mouse IgG (H + L) (Poly-HRP40)	1 mg	\$ 595.00
43R-IG163PH	Goat anti Mouse IgG (H + L) (Poly-HRP40)	50 ug	\$ 250.00
55R-GAMPBLOT	Mouse IgG (H + L) Poly-HRP Blotting Developer Kit	1 kit	\$ 895.00
43R-IGO96PHRP	Goat anti Rabbit IgG (H + L) (Poly-HRP40)	1 mg	\$ 525.00
43R-IG164PH	Goat anti Rabbit IgG (H + L) (Poly-HRP40)	50 ug	\$ 250.00
55R-GARPLOT	Rabbit IgG (H + L) Poly-HRP Blotting Developer Kit	1 kit	\$ 895.00
30R-AH071	Mouse IgG Blocking Reagent	10 ml	\$ 104.50
61R-I169	Mouse anti Human IgG (biotin)	1 mg	\$ 275.00
61R-I167	Mouse anti Human IgG (DY549)	1 mg	\$ 375.00
61R-I165AHRP40	Human IgG (Poly-HRP)	1 mg	\$ 375.00
61R-I164AHRP40	Human IgG (Poly-HRP) Fab'2	500 ug	\$ 425.00
61R-I166AHRP40	Human IgG (Poly-HRP)	50 ug	\$ 145.00
61R-I163AHRP40	Human IgM antibody (Poly-HRP)	1 ml	\$ 425.00
85R-125	PBS (10X Concentrate)	100 ml	\$ 104.50
85R-120	TMB Substrate	30 ml	\$ 104.50
85R-112	Streptavidin Poly-HRP Conjugate Stabilizer	100 ml	\$ 104.50
85R-104	Streptavidin Poly-HRP Conjugate Stabilizer	1 liter	\$ 525.00
65R-S129	Streptavidin protein (DY549)	1 mg	\$ 425.00
85R-127	Substrate Buffer (10X Concentrate)	100 ml	\$ 104.50
85R-123	Stop/Elution Solution	100 ml	\$ 104.50
65R-S124	Streptavidin-Glucose Oxidase	1 mg	\$ 165.00
65R-S126	Streptavidin-Glucose Oxidase (Polymeric 20X)	1 mg	\$ 275.00
65R-S125	Streptavidin-Glucose Oxidase (Oligomeric 5X)	1 mg	\$ 235.00
65R-S103PHRP	Streptavidin Poly-HRP20 Conjugate	1 mg	\$ 451.00
65R-S103PHRP	Streptavidin Poly-HRP20 Conjugate	200 ug	\$ 104.50
65R-S108	Streptavidin Poly-HRP20 Conjugate	50 ug	\$ 375.00
65R-S104PHRP	Streptavidin Poly-HRP40 Conjugate	1 mg	\$ 525.00
65R-S104PHRP	Streptavidin Poly-HRP40 Conjugate	100 ug	\$ 104.50
65R-S112	Streptavidin Poly-HRP40 Conjugate	50 ug	\$ 250.00
65R-S105PHRP	Streptavidin Poly-HRP80 Conjugate	1 mg	\$ 624.75
65R-S105PHRP	Streptavidin Poly-HRP80 Conjugate	100 ug	\$ 104.50
55R-SPBLOT	Streptavidin Poly-HRP Blotting Starter Kit	1 kit	\$ 1,195.00
55R-START01	Streptavidin Poly-HRP ELISA Starter Kit	1 kit	\$ 395.00
55R-START1	Streptavidin Poly-HRP ELISA Starter Kit	1 kit	\$ 595.00
55R-START2	Streptavidin Poly-HRP ELISA Developer Kit	1 kit	\$ 895.00
55R-START3D10	Streptavidin Poly-HRP ELISA Developer Kit	1 kit	\$ 1,395.00
85R-117	TMB Substrate	30 ml	\$ 104.50
85R-119	TMB Weakener	1 liter	\$ 375.00
85R-108	Universal Casein Diluent/Blocker	1 liter	\$ 287.50
85R-108	Universal Casein Diluent/Blocker	100 ml	\$ 104.50
55R-PHRPX-SA	Streptavidin Poly-HRP (+ 100ml PHRPX)	1 pack	\$ 313.50
85R-126	Wash Buffer (10X Concentrate)	100 ml	\$ 104.50
85R-202	High Load Streptavidin-PolyHRP Conjugate	1 ml	\$ 295.00
43R-MIGHRP80	Mouse anti Human IgG (Poly-HRP80)	1 mg	\$ 595.00