

Enzyme Conjugates

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
Enzyme conjugates	See Materials for specific product.	See Materials for specific product.	All except S921: <ul style="list-style-type: none"> • ≤-20°C • Desiccate S921: <ul style="list-style-type: none"> • 2-6°C • DO NOT FREEZE 	See Materials for specific product.

Introduction

Enzyme conjugates are employed in enzyme-linked immuno-sorbent assays (ELISAs), blotting techniques, *in situ* hybridizations, immunocytochemistry, immunohistochemistry, and tyramide signal amplification (TSA). Molecular Probes' enzyme conjugates (Tables 2 and 3) provide very sensitive detection, and our selection of fluorogenic substrates ranges in color from blue to red (Table 4).

We also prepare a biotin-XX conjugate of peroxidase, which has been used in applications such as retrograde tracing, dye coupling, and fate mapping (Table 3). Biotin-XX-labeled peroxidase may also have utility as a retrograde tracer and can be detected by its own activity or with avidin or streptavidin conjugates. Additionally, we offer horseradish peroxidase conjugates of the high-affinity rabbit anti-fluorescein/Oregon Green® IgG fraction that can be used to considerably amplify the fluorescence of fluorescein/Oregon Green® dye-labeled probes (Table 2).

Table 2. Enzyme conjugates of antibodies.

Host	Antigen	Antibody form *	Horseradish peroxidase	Alkaline phosphatase	β-Lactamase
Goat	mouse IgG (H+L)	IgG	G21040	G21060	G31567
Goat	mouse IgG (H+L)	F(ab') ₂	G21453	G21452	
Goat	mouse IgG ₁ (γ ₁)	IgG ₁	A10551		
Goat	mouse IgG _{2a}	IgG	A10685		
Goat	rabbit IgG (H+L)	IgG	G21234	G21079	G31568
Goat	rabbit IgG (H+L)	F(ab') ₂	A10547	G21456	
Goat	rat IgG (H+L)	IgG	A10549	A10546	
Goat	rat IgG (H+L)	F(ab') ₂	A10548		
Rabbit	mouse IgG (H+L)	IgG	R21455		
Rabbit	goat IgG (H+L)	IgG	R21459	R21458	
Rabbit	fluorescein/Oregon Green 488	IgG	A21253		

* IgG, whole antibody IgG fraction, or F(ab')₂ fragment of IgG.

Table 3. Enzyme conjugates of avidins, protein G, or biotin.

Protein or biotin	Horseradish peroxidase	Alkaline phosphatase	β -Galactosidase	β -Lactamase
Streptavidin	S911	S921	S931	S31569
NeutrAvidin™	A2664			
Protein G	P21041			
Biotin-XX	P917			

Table 4. Characteristics of enzyme substrates.

Enzyme	Substrate	Ex/Em*	Notes
Alkaline phosphatase	6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP; D6567)	358/455	<ul style="list-style-type: none"> Monophosphorylated (removal of one phosphate results in product's fluorescence) pK_a 4.7, nonfluorescent until phosphate is hydrolyzed
	methylumbelliferyl phosphate (MUP; M6491, M8425)	360/449	<ul style="list-style-type: none"> Monophosphorylated (removal of one phosphate results in product's fluorescence) pK_a 7.8, nonfluorescent until phosphate is hydrolyzed
	fluorescein diphosphate (FDP, F2999)	490/514	<ul style="list-style-type: none"> Diphosphorylated (removal of two phosphates results in product's fluorescence/ absorbance) pK_a 6.4, nonfluorescent and non-absorbing in visible wavelength until phosphate is hydrolyzed
	DDAO phosphate (D6487)	646/659	<ul style="list-style-type: none"> Monophosphorylated (removal of one phosphate results in an excitation maxima shift of >200 nm) pK_a 6.0, unhydrolyzed substrate has Ex/Em maxima at 478/628 nm
	ELF [®] 97 phosphate (E6588, E6589)	345/530	<ul style="list-style-type: none"> Product precipitates at the site of the reaction Following enzymatic cleavage the substrate yields a precipitate with a very large Stokes shift (>180 nm) and excellent photostability
Horseradish peroxidase	Amplex [®] Red reagent (A12222, A22177)	563/587	<ul style="list-style-type: none"> Amplex[®] Red reagent has greater stability, yields less background and produces a red-fluorescent product that is more readily detected than the similar reduced methylene blue derivatives commonly used for colorimetric determination Amplex[®] Red reagent reacts with H₂O₂ in a 1:1 stoichiometric ratio to produce a brightly fluorescent and strongly absorbing reaction product
	Amplex [®] UltraRed reagent (A36006)	568/581	<ul style="list-style-type: none"> Fluorescence of the Amplex[®] UltraRed reagent exhibits greater stability in the presence of H₂O₂ or thiols (such as DTT) and shows reduced sensitivity to pH when compared to Amplex[®] Red reagent Amplex[®] UltraRed reagent reacts with H₂O₂ in a 1:1 stoichiometric ratio to produce a brightly fluorescent and strongly absorbing reaction product
β -lactamase	Fluorocillin™ Green 495/525 reagent (F33952) †	495/525	<ul style="list-style-type: none"> Nonfluorescent and nonabsorbing at visible wavelengths prior to cleavage by β-lactamase More sensitive and less prone to hydrolysis than commonly used colorimetric reagents Requires no toxic cofactors like H₂O₂ and will work in the presence of EDTA, many detergents, salts, and azide

* Invitrogen also offers Fluorocillin™ Green 345/530 β -lactamase substrate (F33951), but this substrate does not work with TEM-1 β -lactamase or any conjugates of this recombinant enzyme, including G31567, G31568, and S31569. It has, however, been shown to be an effective substrate for other β -lactamases.

Materials

Peroxidase Conjugates

The peroxidase conjugates of streptavidin (Cat. no. S911), protein G (Cat. no. P21041), and whole antibodies (Cat. nos. A10549, A10551, A21253, G21040, G21234, R21455, R21459) are supplied in unit sizes of 1 mg (or 0.5 mg, Cat. no. A21253) of protein as lyophilized powder, including some buffer salts. When stored desiccated at $\leq -20^{\circ}\text{C}$, the lyophilized powder is stable for at least one year. These products may be reconstituted in 1 mL (or 0.5 mL, Cat. no. A21253) of phosphate-buffered saline (PBS), pH 7.2, to yield 1 mg/mL stock solutions.

The peroxidase conjugate of an antibody F(ab')₂ fragment (Cat. nos. A10547, A10548, G21453) is supplied in a unit size of 0.5 mg of protein as lyophilized powder, including some buffer salts. This product may be reconstituted in 0.5 mL of PBS, pH 7.2, to yield a 1 mg/mL stock solution. Store solution at 2–6°C with the addition of thimerosal to a final concentration of 0.02%. For prolonged storage after reconstitution, add glycerol to a final concentration of 50% (v/v), aliquot, and store at $\leq -20^{\circ}\text{C}$. When stored properly, the solution is stable for approximately three months.

The biotin-XX-labeled peroxidase conjugate (Cat. no. P917) is supplied lyophilized in a unit size of 10 mg. When stored desiccated at $\leq -20^{\circ}\text{C}$, the lyophilized powder is stable for at least one year. Solutions may be prepared by dissolving the powder in PBS or other suitable buffer. Store solutions at 2–6°C with the addition of thimerosal to a final concentration of 0.02%.

Alkaline Phosphatase Conjugates

The alkaline phosphatase (AP) conjugate of streptavidin (Cat. no. S921) is supplied in a unit size of 500 μL of a 2 mg/mL solution in 30 mM triethanolamine (TEA), 3 M NaCl, 1 mM MgCl₂, 0.1 mM ZnCl₂, pH 7.6, containing 1% bovine serum albumin (BSA) and 2 mM sodium azide. This product is stable for at least six months when stored undiluted at 2–6°C. **Do not freeze.**

The conjugates of whole antibodies (Cat. nos. A10546, G21060, G21079, R21458) are supplied in unit sizes of 1 mg of protein as lyophilized powder from 0.5 mL of a 2 mg/mL solution in 50 mM Tris (pH 8.0), 30 mM trehalose, and 100 mM NaCl. These conjugates may be reconstituted in 0.5 mL of deionized water to make 2 mg/mL stock solutions.

The alkaline phosphatase conjugates of antibody F(ab')₂ fragments (Cat. nos. G21452, G21456) are supplied in unit sizes of 0.5 mg of protein as a lyophilized powder from 250 μL of 2 mg/mL solutions in 50 mM Tris (pH 8.0), 30 mM trehalose, and 100 mM NaCl. These conjugates may be reconstituted in 250 μL of deionized water to make 2 mg/mL stock solution. The lyophilized conjugates are stable for at least 6 months when stored desiccated at $\leq -20^{\circ}\text{C}$. Store stock solutions at 2–6°C with the addition of sodium azide to a final concentration of 2 mM. When stored properly, solutions are stable for at least 3 months.

β -Galactosidase–Streptavidin Conjugate

The β -galactosidase conjugate of streptavidin (Cat. no. S931) is supplied in a unit size of 1 mg as lyophilized powder from 0.5 mL of a 2 mg/mL solution in 30 mM TEA, pH 7.6, containing 0.5% BSA. The product may be reconstituted in 0.5 mL of distilled water to yield a 2 mg/mL stock solution.

The conjugate is stable for at least one year when stored desiccated at $\leq -20^{\circ}\text{C}$. Store solutions at 2–6°C with the addition of sodium azide to a final concentration of 2 mM. When stored properly, solutions are stable for approximately three months.

β -Lactamase Conjugate

The β -lactamase conjugate of streptavidin (Cat. no. S31569) and whole antibodies (Cat. nos. G31567 and G31568) are supplied in a unit size of 0.5 mg of protein conjugate as lyophilized powder from PBS pH 7.2. The lyophilized β -lactamase conjugates are stable for at least 6 months when stored desiccated at $\leq -20^{\circ}\text{C}$.

Reconstitute the conjugate by adding 0.5 ml of deionized water that includes 2–5 mM sodium azide directly to the vial, to create a 1 mg/ml stock solution. Alternatively, reconstitute β -lactamase conjugate by adding 0.5 ml of a 50% deionized water, 50% glycerol (v/v) solution containing 2–5 mM sodium azide. The 50% glycerol stock should be divided into aliquots and stored at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles. When stored properly, solutions are stable for approximately three months.

Properties

Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step eliminates any protein aggregates that may form during storage, and reduces nonspecific background staining.

Peroxidase Conjugates

The specific activity of the biotinylated horseradish peroxidase conjugate is approximately 100–300 units/mg; one unit is defined as the amount of enzymatic activity required to produce 1.0 mg of purpurogallin from pyrogallol in 20 seconds at 21°C , pH 6.0.

The specific activity of the streptavidin, NeutrAvidin™, antibody, or protein G conjugates of horseradish peroxidase is approximately 50–150 units/mg. The molecular weight of horseradish peroxidase itself is approximately 44,000 daltons. For ELISAs using the streptavidin, NeutrAvidin™, antibody, or protein G conjugates working dilutions of 1:500 to 1:2,000 from 1 mg/mL solutions are suitable. For the biotin-XX conjugate, use a working dilution of 1:2,000 to 1:5,000 from a 1 mg/mL solution.

Alkaline Phosphatase Conjugates

The specific activity of the alkaline phosphatase conjugates is approximately 150–350 units/mg. One unit is defined as the amount of enzymatic activity required to hydrolyze 1.0 micromole of *p*-nitrophenyl phosphate to *p*-nitrophenol and inorganic phosphate in 60 seconds at 21°C , pH 10.

 β -Galactosidase–Streptavidin Conjugate

The specific activity of the β -galactosidase conjugate of streptavidin is about 150 units/mg when assayed at 21°C . One enzyme unit can hydrolyze one micromole of substrate, 2-nitrophenyl β -D-galactopyranoside (ONPG), per minute in 100 mM potassium phosphate, pH 7.0, 1 mM MgCl_2 , 0.1 M 2-mercaptoethanol, 2.3 mM ONPG.

 β -Lactamase Conjugate

The specific activity of the β -lactamase conjugates is approximately 150–300 units/mg; one unit is defined as the amount of enzyme activity required to hydrolyze one micromole of ampicillin per minute at pH 7.5 at 21°C .

Fluorogenic Substrates

Invitrogen offers a number of fluorogenic substrates for use with our enzyme conjugates. Table 4 gives the spectral information for each substrate along with brief application guidelines.

References

1. J Exp Zool 255, 323 (1990).

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
A21253	anti-fluorescein/Oregon Green [®] , rabbit IgG fraction, horseradish peroxidase conjugate.....	0.5 mg
A2664	avidin, NeutrAvidin™, horseradish peroxidase conjugate.....	1 mg
F21452	F(ab') ₂ fragment of goat anti-mouse IgG (H+L), alkaline phosphatase conjugate.....	0.5 mg
F21453	F(ab') ₂ fragment of goat anti-mouse IgG (H+L), horseradish peroxidase conjugate.....	0.5 mg
F21456	F(ab') ₂ fragment of goat anti-rabbit IgG (H+L), alkaline phosphatase conjugate.....	0.5 mg
A10547	F(ab') ₂ fragment of goat anti-rabbit IgG (H+L), horseradish peroxidase conjugate.....	0.5 mg
A10548	F(ab') ₂ fragment of goat anti-rat IgG (H+L), horseradish peroxidase conjugate.....	0.5 mg
G21040	goat anti-mouse IgG (H+L), horseradish peroxidase conjugate.....	1 mg
G21060	goat anti-mouse IgG (H+L), alkaline phosphatase conjugate.....	1 mg
G21079	goat anti-rabbit IgG (H+L), alkaline phosphatase conjugate.....	1 mg
G21234	goat anti-rabbit IgG (H+L), horseradish peroxidase conjugate.....	1 mg
G31567	goat anti-mouse IgG (H+L), β-lactamase TEM-1 conjugate *0.5 mg net protein*.....	0.5 mg
A10551	goat anti-mouse IgG ₁ (γ ₁), horseradish peroxidase conjugate.....	0.5 mg
G31568	goat anti-rabbit IgG (H+L), β-lactamase TEM-1 conjugate *0.5 mg net protein*.....	0.5 mg
A10546	goat anti-rat IgG (H+L), alkaline phosphatase conjugate.....	0.5 mg
A10549	goat anti-rat IgG (H+L), horseradish peroxidase conjugate.....	0.5 mg
P21041	protein G, horseradish peroxidase conjugate.....	1 mg
P917	peroxidase from horseradish, biotin-XX conjugate.....	10 mg
R21455	rabbit anti-mouse IgG (H+L), horseradish peroxidase conjugate.....	1 mg
R21458	rabbit anti-goat IgG (H+L), alkaline phosphatase conjugate.....	1 mg
R21459	rabbit anti-goat IgG (H+L), horseradish peroxidase conjugate.....	1 mg
S31569	streptavidin, β-lactamase TEM-1 conjugate *0.5 mg net protein*.....	0.5 mg
S911	streptavidin, horseradish peroxidase conjugate.....	1 mg
S921	streptavidin, alkaline phosphatase conjugate *2 mg/mL*.....	0.5 mL
S931	streptavidin, β-galactosidase conjugate.....	1 mg

Contact Information

Molecular Probes, Inc.

29851 Willow Creek Road
Eugene, OR 97402
Phone: (541) 465-8300
Fax: (541) 335-0504

Customer Service:

6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338
Fax: (541) 335-0305
probesorder@invitrogen.com

Toll-Free Ordering for USA:

Order Phone: (800) 438-2209
Order Fax: (800) 438-0228

Technical Service:

8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 335-0353
Toll-Free (800) 438-2209
Fax: (541) 335-0238
probestech@invitrogen.com

Invitrogen European Headquarters

Invitrogen, Ltd.
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Phone: +44 (0) 141 814 6100
Fax: +44 (0) 141 814 6260
Email: euroinfo@invitrogen.com
Technical Services: eurotech@invitrogen.com

**For country-specific contact information,
visit www.invitrogen.com.**

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