



SureLINK™ Conjugation Kits

On the prowl for easy and reliable protein labeling tools?

Try SureLINK Conjugation Kits!

SureLINK Conjugation Kits provide a fast and reliable approach to label your proteins or antibodies. All necessary reagents for labeling proteins with either alkaline phosphatase (AP) or horseradish peroxidase (HRP) are provided. Resultant conjugates are ideal for use in a variety of applications, such as ELISA, Western blotting and immunohistochemistry.

Sure to work everytime

Achieve consistent conjugation results everytime. Robust protocols are not sensitive to minor variations in protein concentrations and can be modified to suit your application.

Fast protocol requires little hands-on time

Label your protein with HRP in just 90 minutes or with AP in 3 hours. Start-to-finish, hands-on time is less than 20 minutes. No lengthy purification or quantitation is required. Detection can begin immediately.

Convenient kits make labeling easy

Successful protein labeling is made easy with comprehensive, optimized kits. Ready-to-use reagents and stabilized, activated enzymes are supplied in reaction-size vials. Enzymes are preactivated and buffers are ready to use. Detailed manuals guide first-time and experienced users through conjugation.

Versatile kits and tools fit every need

Conjugation-ready enzymes, unique linkers and complete kits are offered for a variety of protein labeling needs. Several reaction sizes make it easy to conjugate various amounts of protein. Enzymes are also available separately for use with your own protocols and buffers.

No need to look further. You've just found conjugation kits that deliver outstanding assay results! Try SureLINK and SEE MORE with KPL!



Where Better Science Begins

SureLINK™ Conjugation Kits

SureLINK AP Conjugation Kits

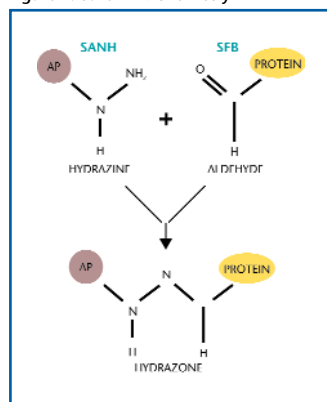
Assured performance

Take the guesswork out of protein labeling with SureLINK AP Conjugation Kits. Novel cross-linking reagents SANH and SFB form the basis of the coupling reaction (Figure 1). The reaction between aldehyde and hydrazine groups forms a hydrazone bond that is highly selective, resulting in AP conjugates that are stable for at least six months.

SureLINK AP Kits overcome many of the limitations of other conjugation methods based on glutaraldehyde and maleimide/thiol (Table 1).

- No reducing agents are required to stabilize the bond. Therefore, disulfide bonds are not reduced and proteins are not denatured.
- Modified proteins are stable and can be prepared in advance.
- Unwanted cross-linking is virtually eliminated by the highly specific linkage.

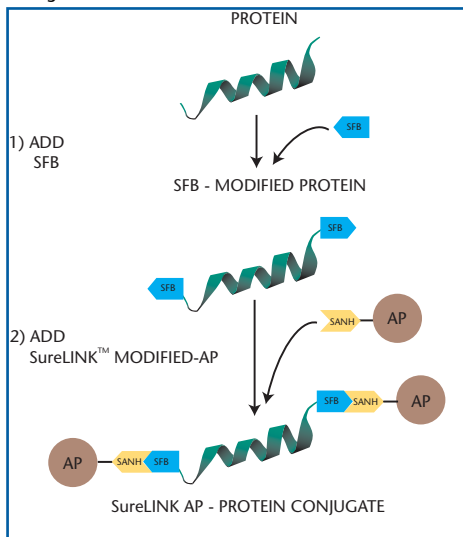
Figure 1: SureLINK Chemistry



Fast and easy protocol

SureLINK AP Conjugates can be produced with twenty minutes hands-on time and three hours elapsed time. Several hours may be saved versus the traditional maleimide/thiol method (Table 1). The two-step SureLINK protocol (Figure 2) is also easy to perform.

Figure 2: SureLINK AP Protocol



- Modified AP is ready for conjugation.
- Proteins do not need to be desalted or dialyzed.
- Kits include ready-to-use buffers, including storage buffer.
- Detailed protocol is provided.

Minimal optimization

SureLINK AP Kits utilize heterobifunctional cross-linkers that minimize the risk of nonspecific interactions. In contrast, glutaraldehyde methods must be optimized in order to reduce polymerization and self-conjugation. SureLINK kits decrease the time required for optimization of the conjugation reaction.

Versatile and convenient

SureLINK AP Conjugation Kits can be used to conjugate a range of protein sample sizes. Choose the kit that best matches your protein labeling needs.

- Three 0.5-mg samples can be conjugated with the larger kit or three 0.1-mg samples with the smaller kit.
- Modified-AP is supplied in convenient, reaction-size vials. It can be purchased separately and is compatible with other protocols and reagents.

Great for immunodetection

Using SureLINK AP Kits is a great way to generate monoclonal antibody conjugates for ELISA, Western blotting, and immunohistochemistry (See Figures 3, 4 and 5). Streptavidin can be labeled when greater sensitivity is required. Moreover, a variety of proteins in a range of sizes can be conjugated to AP using the SureLINK AP Conjugation Kits.

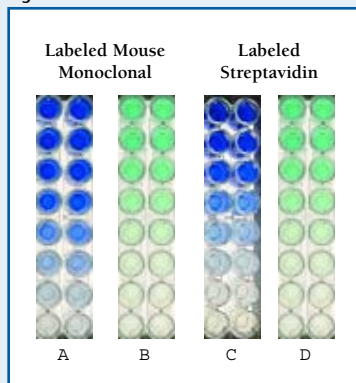
Table 1: Comparison of AP Conjugation Protocols

Feature	SureLINK	Maleimide/Thiol	Glutaraldehyde
Protein Preparation	Modify protein with SFB	Reduce thiols (90 min.) Desalt and fractionate. Determine protein concentrations.	Not required
Enzyme Preparation	Not required. Ready to conjugate.	Activate AP with maleimide.	Dialyze
Conjugation Reaction	2 hours	2 hours	0.5 – 4 hours
Conjugation Preparation	Add AP Storage Buffer	Dialyze. Prepare storage buffer and add.	Dialyze. Prepare storage buffer and add.
Total Steps	3	6	2
Total Time	3 hours	>5 hours	2 – 4.5 hours
Hands-on Time	20 minutes	~3 hours	20 minutes
Protocol Optimization	Not required	Required	Required

Immunodetection with SureLINK Conjugates

SureLINK AP and HRP kits were used to label a monoclonal antibody (mouse anti-goat IgG), a polyclonal antibody and streptavidin. Resulting conjugates were used in ELISA (Figure 3), Western blotting (Figure 4) and immunohistochemistry (Figure 5) assays using KPL's substrates and reagents. Results demonstrate that SureLINK conjugates enable sensitive, reproducible performance in protein detection applications.

Figure 3

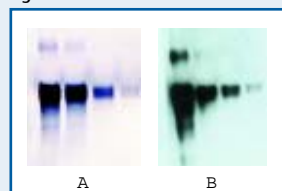


ELISA

Columns A and B: Wells were coated using serial dilutions of goat IgG. SureLINK AP- or HRP-labeled anti-goat IgG was added to each well. BluePhos® Substrate (A) or ABTS® Substrate (B) was used for detection.

Columns C and D: Wells were coated with serial dilutions of mouse IgG. Biotinylated goat anti-mouse IgG was added, followed by SureLINK AP- or HRP-labeled streptavidin. BluePhos Substrate (C) or ABTS Substrate (D) was used for detection.

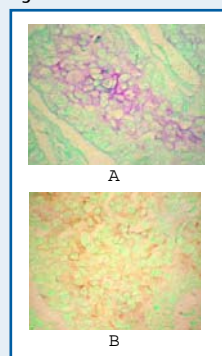
Figure 4



Western Blotting

Goat IgG was separated by SDS-PAGE and transferred to nitrocellulose membrane. SureLINK Kits were used to label mouse anti-goat IgG with either AP (A) or HRP (B). Standard Western blotting procedures were performed. LumiGLO® Chemiluminescent Substrate (A) or BCIP/NBT Substrate (B) was used for detection.

Figure 5



Immunohistochemistry

Human epithelial membrane antigen clone 29 was detected in formalin-fixed, paraffin-embedded normal human breast tissue using SureLINK AP goat anti-mouse IgG conjugate and HistoMark® RED (A) or with a biotin/streptavidin system using SureLINK streptavidin-HRP conjugate and DAB Reagent Set (B).

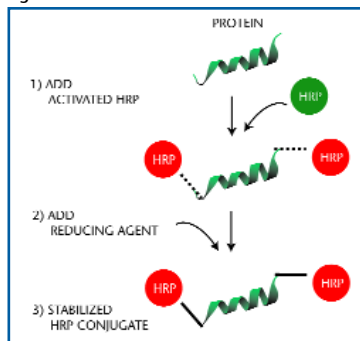
SureLINK HRP Conjugation Kits

Sure results and stable conjugates

SureLINK HRP Conjugation Kits are based on well-established periodate chemistry. The process yields consistent and reproducible protein conjugates.

- HRP is activated with periodate to form reactive aldehyde groups. These groups readily couple with primary amines present on proteins at basic pH to form a Schiff base.
- Reduction of the Schiff base linkage stabilizes the conjugates. Conjugates prepared using this chemistry are stable for at least six months.

Figure 6: SureLINK HRP Protocol



Fast and easy protocol

SureLINK HRP Kits contain all reagents required to label your protein or antibody with HRP. HRP is ready to use and requires no activation

or purification. The protocol is simple and easy to perform (Figure 6). The reaction takes less than 20 minutes hands-on time and is complete in 90 minutes.

Flexibility and convenience

Conjugate a range of protein sample sizes with SureLINK HRP Kits. Choose the kit that best matches your protein labeling needs.

- Six, 1.0-mg samples can be conjugated with the larger kit or six, 0.1-mg samples with the smaller kit. Two, 0.1-mg protein samples can be conjugated with the trial kit.
- Activated HRP is supplied in convenient, reaction-size vials. It is also available separately for use with your protocol and reagents.

Compatible with KPL reagents

Conjugates prepared with SureLINK HRP Conjugation Kits can be used in many immunodetection assays including ELISA, Western blotting and immunohistochemistry. The results in Figures 3, 4 and 5 demonstrate the use of SureLINK HRP conjugates in chemiluminescent and colorimetric detection formats using KPL substrates, blockers, and washing solutions.

Ordering Information

Catalog#	Description	Size
	SureLINK™ AP Conjugation Kits Each kit includes SureLINK Modified AP, AP Modification Buffer, SFB, AP Conjugation Buffer and AP Storage Buffer.	
85-00-02	SureLINK AP Conjugation Kit	3 x 0.5 mg rxn.
85-00-01	SureLINK AP Conjugation Kit	3 x 0.1 mg rxn.
	SureLINK HRP Conjugation Kits Each kit includes SureLINK Activated HRP, HRP Conjugation Buffer, Reducing Reagent and HRP Storage Buffer.	
84-00-02	SureLINK HRP Conjugation Kit	6 x 1 mg rxn.
84-00-01	SureLINK HRP Conjugation Kit	6 x 0.1 mg rxn.
84-00-03	SureLINK HRP Conjugation Kit	2 x 0.1 mg rxn.
	SureLINK Activated HRP and Modified AP	
84-01-02	SureLINK Activated HRP	1.5 mg
84-01-01	SureLINK Activated HRP	0.3 mg
85-01-02	SureLINK Modified AP*	1.0 mg
85-01-01	SureLNK Modified AP*	0.2 mg
80-02-04	SFB	100 mg
80-02-02	SFB	20 mg (4 x 5 mg)

*SureLINK Modified AP must be used with SFB linker for successful conjugation of proteins.

Discover KPL's wide selection of sensitive substrates and reagents designed to give you high signal-to-noise in your protein detection assays.

See More with KPL!
Better conjugates
make better assays.
Try SureLINK products
and SEE MORE!

Pictured on front and to right:
Jaguar cub



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BluePhos, LumiGLO and HistoMARK are registered trademarks of KPL.
ABTS is a registered trademark of Boehringer Mannheim, GMBH.
SureLINK AP Conjugation Kits and Modified AP are produced with components protected by U.S. Patent Numbers 6,800,728, 5,679,778, 5,420,285, 5,753,520 and 5,206,370.
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Frequently Asked Questions

What amount of protein or antibody can be conjugated with SureLINK HRP and AP kits?

SureLINK HRP Kits have been optimized for conjugating antibody or protein within the range of 0.05 mg to 1 mg, and SureLINK AP Kits within the range of 0.05 to 0.5 mg.

What advantages are offered by the SANH and SFB heterobifunctional linkers used in the SureLINK AP Kits?

- Very selective conjugation reaction with minimal polymer formation.
- No need to stabilize the hydrazone bond, minimizing the possibility of compromising the biological activity of the conjugate.

How stable are the resultant SureLINK conjugates and how should they be stored to ensure optimal stability and performance?

Conjugates prepared using SureLINK HRP and AP Kits are stable when stored at 4° C for at least 6 months.

Can SureLINK Conjugation Kits be utilized with other protein and oligonucleotides?

Yes, so long as the target biomolecules contain a primary amine group that can be readily modified with SFB (AP kits) or is compatible with periodate chemistry (HRP kits). Oligonucleotides need to be synthesized with a 3' or 5' amine modification.

Gaithersburg, MD
Phone: 800.638.3167/301.948.7755
Fax: 301.948.0169
www.kpl.com



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