



Overview of Solulink Products

June 2011

Who we are

Established in 2003, Solulink develops, patents, manufactures, and sells consumables to over 1,000 customers in life science, diagnostic, and pharmaceutical markets.

Opportunities: Customers want diagnostics and assays to detect disease earlier and to run more tests at the same time. Aging worldwide baby boomer population drives the need for more accurate, earlier, and faster detection diagnostics for everything from cancer to arthritis.

Solutions: Solulink has invented a proprietary molecule that is especially good at linking (conjugating) detection and identification components onto all types of antibodies, peptides, DNA, RNA, and proteins, which speeds assay development and provides new capabilities. Solulink has been validated by customers at prestigious pharmaceutical, biotechnology, and clinical research institutions and university laboratories worldwide.

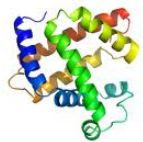
Kits and reagents

- Biotinylation kits and reagents
- Magnetic beads and agarose
- Antibody labeling kits
- Crosslinking kits and reagents
- Western blot kits
- Custom conjugation services

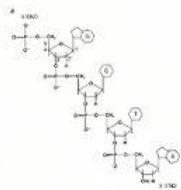
Many linking possibilities...



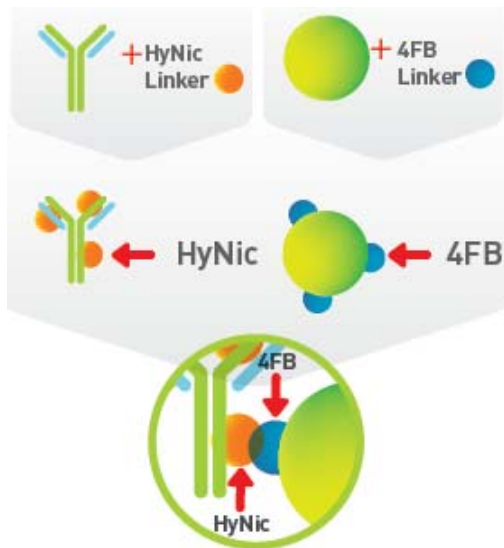
Antibody (IgG)



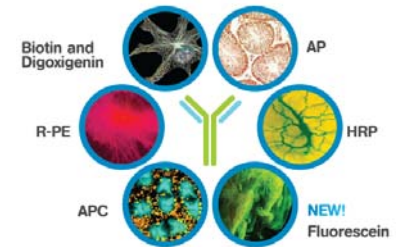
Proteins



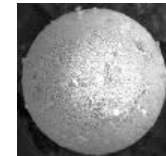
Oligos



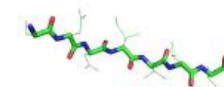
Two Linker System



Enzymes, dyes, biotin, DIG



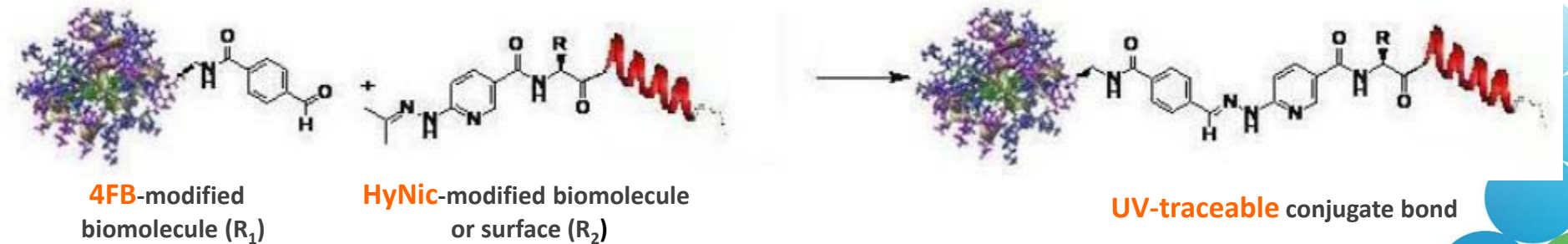
Magnetic beads and surfaces



Peptides

Bioconjugation

- **Bioconjugation** – The linking of two biomolecules to form a hybrid, the bioconjugate.
- **Labeling (Modification)** – Adding a linker or dye to an unmodified biomolecule
- **Conjugation (Crosslinking)** – Linking 2 modified biomolecules



What makes our chemistry **unique?**

- **Kinetics:** Fast conjugation < 30 min with new catalyst
- **Reproducible:** Controllable, specific covalent linking
- **Efficient:** Higher yields, easier purification, better stoichiometry
- **Quantifiable:** UV-traceable conjugations/linkers
- **Linker stability:** Stable on modified biomolecules and surfaces
- **Conjugate stability:** Thermo-stable to PCR temperatures; pH stable 2.5-10
- **Bio-orthogonal:** 1 + 1 = 3 Retains inherent biological function of biomolecules
- **Versatile:** One technology for conjugation and immobilization of all biomolecules
- **Variety of linkers available:** PEG4, disulfide cleavable
- **Gentle:** Preserves antibody, biomolecule functionality
- **Scalable:** Micrograms to grams to kilos



solulink™



1. Biotinylation

GET BETTER RESULTS.
GUARANTEED.



7
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Why biotinylate?

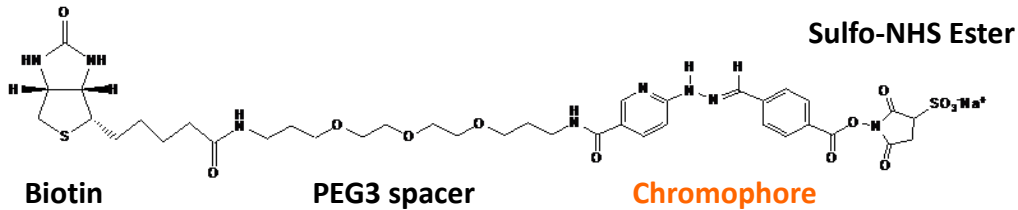
- Applications (4 primary uses for biotinylated antibodies)

The biotin/streptavidin system is useful in several types of assays; biotinylated antibodies (90% of biotinylation projects) allow replacement of anti-species secondary antibodies

1. **Western blotting** (biotinylated primary Ab + S-AP or S-HRP or S-PE)
2. **Immobilization** (biotinylated antibodies attached to streptavidin-coated beads—pull out rare cells or antigens from a mixture)
3. **Immunohistochemistry (IHC)** (biotinylated antibody detects rare cells or antigens on slide + S-AP or S-HRP or S-PE for signal)
4. **ELISA** (polyclonal Ab attached to plate. Add antigen. Add biotinylated antibody. Add S-AP or S-HRP or S-PE)

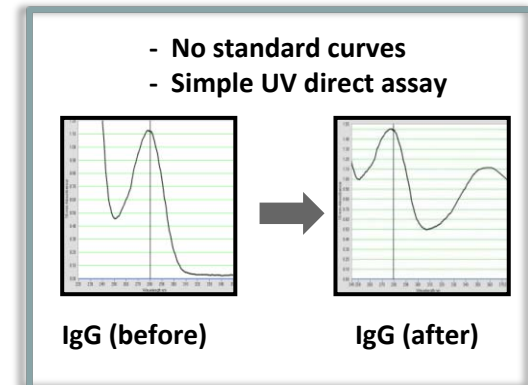
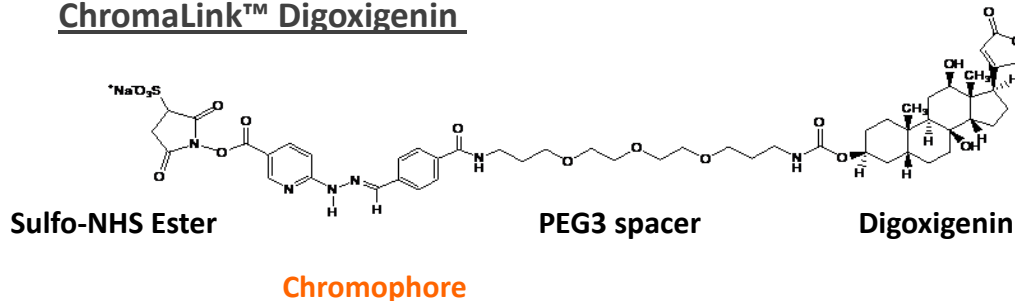
Solulink biotinylation

ChromaLink™ Biotin



- Reproducible biotin labeling
- NO HABA ASSAY!

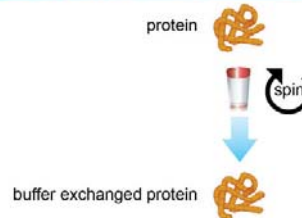
ChromaLink™ Digoxigenin



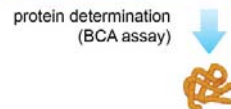
Advantage of ChromaLink™ reagents:
Simply measure A_{280} and A_{354} to determine antibody concentration and # labels/antibody

Biotinylation protocol

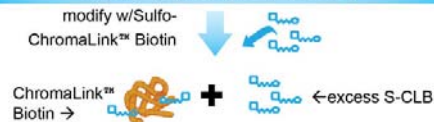
1. First Buffer Exchange (10 minutes)



2. Protein Determination Assay (30 minutes)



3. Biotinylate (90 minutes)



4. Desalting (10 minutes)

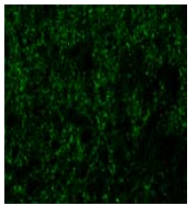


5. Quantify By UV (20 minutes)

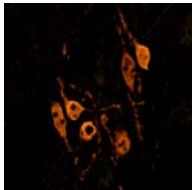


Multi-Color Immunohistochemistry with 3 Mouse mAbs

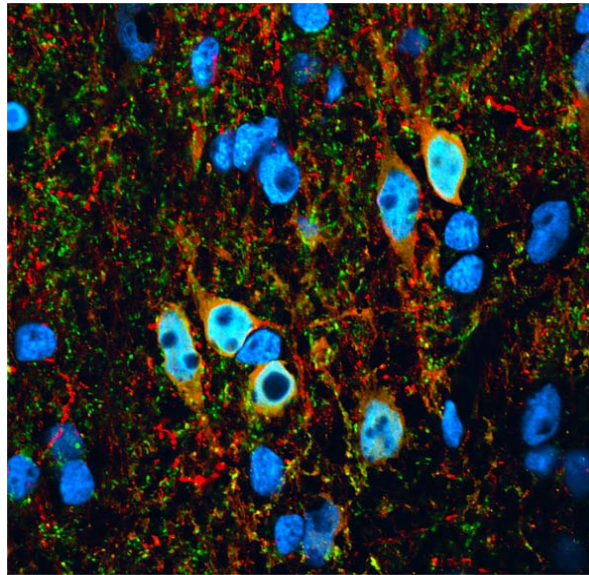
Synaptotagmin (biotin MAB4364)
+
Streptavidin-NL493



Calbindin (unconjugated MAB3320)
+
anti-mouse-NL637

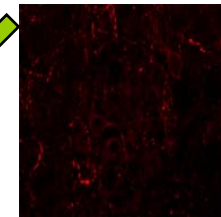


4-color merged image

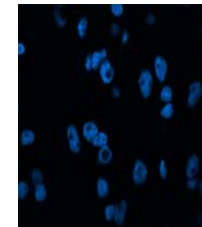


Alex Kaluzhny, PhD, R&D Systems

CART (DIG-MAB163)
+
anti-DIG Rhodamine Red X



DAPI
(nuclear counterstain)



Target 1: Detected by unconjugated antibody, secondary antibody-DYE used

Target 2: Detected by primary antibody-biotin, visualized with streptavidin-DYE (streptavidin binds to the biotin)

Target 3: Detected by primary antibody-DIG, anti-DIG-DYE secondary antibody is used

ChromaLink™ Biotin One-Shot Antibody Labeling Kit

Biotinylate & Quantify Antibody Labeling with 1 Reagent

- The One-Shot Kit features:
 - One 100 µg antibody biotinylation
 - >60% yield in 2 hours
 - One scan traceability
 - One reagent convenience



Top-Selling Biotinylation Products

ChromaLink™ Biotin Labeling Kit

ChromaLink™ One-Shot Antibody Labeling Kit



2. Magnetic Beads and Agarose



NanoLink™ Magnetic Beads

(0.8 μm size, non-uniform)

streptavidin coated

4FB coated

amino coated

MagnaLink™ Magnetic Beads

(2.8 μm size, uniform)

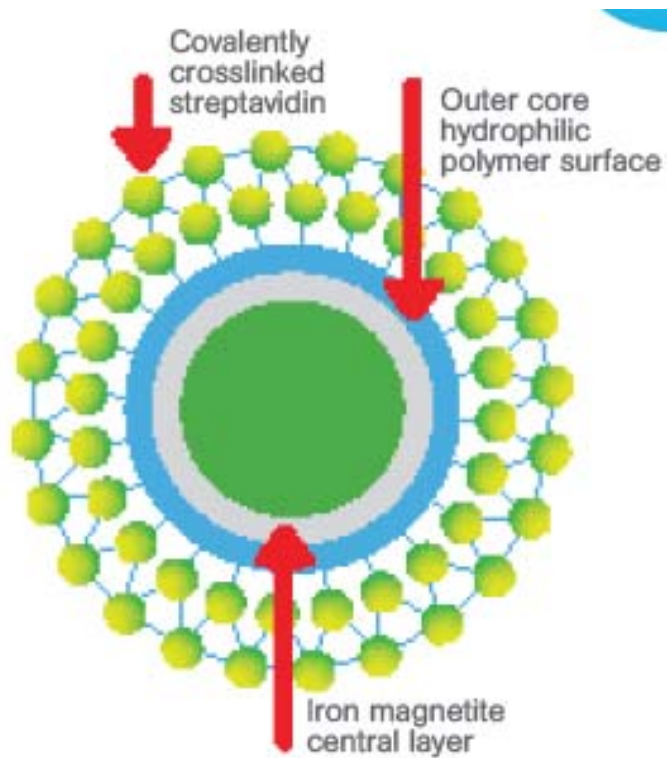
streptavidin coated

4FB coated

amino coated

Magnetic beads

NanoLink™ Streptavidin Magnetic Beads



- Advantages
 - Cost effective
 - Highest biotin binding capacity
- Major application:
 - Capture and immobilization of biotinylated biomolecules

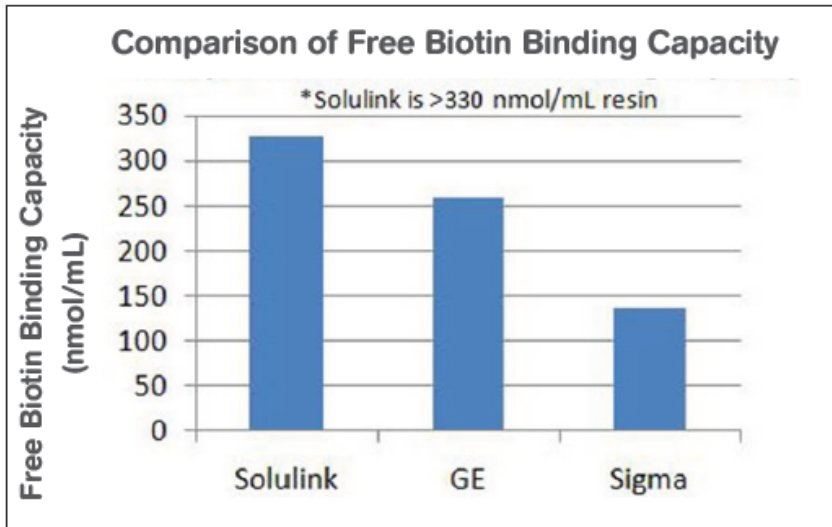
NanoLink™ Magnetic Beads vs. the competition

Molecule	NanoLink (0.8 μm) Binding	Competitor's (1 μm) binding
Free biotin	>14,000 pmol/mg	>1,300 pmol/mg
Biotinylated oligo (23-mer)	>2.5 nmol/mg	NA
Biotinylated IgG (3 biotins per IgG)	250 $\mu\text{g}/\text{mg}$	20 $\mu\text{g}/\text{mg}$

MagnaLink™ Magnetic Beads vs. the competition

Molecule	MagnaLink (2.8 μm) Binding	Competitor's (2.8 μm) binding
Free biotin	>12,000 pmol/mg	650–900 pmol/mg
Biotinylated oligo (23-mer)	>0.8 nmol/mg	NA
Biotinylated IgG (4 biotins per IgG)	112.6 $\mu\text{g}/\text{mg}$	5–10 $\mu\text{g}/\text{mg}$

Streptavidin Agarose Ultra Performance™ (NEW)



Applications

- Immobilization of biotinylated biomolecules or drugs
- Immunoprecipitation, CHIP, cell capture, and others
- Cell-surface labeling with biotinylation reagents, followed by precipitation with streptavidin
- Recovery of biotinylated DNA for dideoxy sequencing

BeadLink™ Kits (NEW)

- Link antibodies directly to magnetic beads
- Modify your antibody with the HyNic crosslinker, which attaches to 4FB-modified beads—all provided in the kit

● **NanoLink™ BeadLink™ Kit**

● **MagnaLink™ BedLink™ Kit**

Top-Selling Bead & Agarose Products

NanoLink™ Streptavidin Magnetic Beads

MagnaLink™ Streptavidin Magnetic Beads

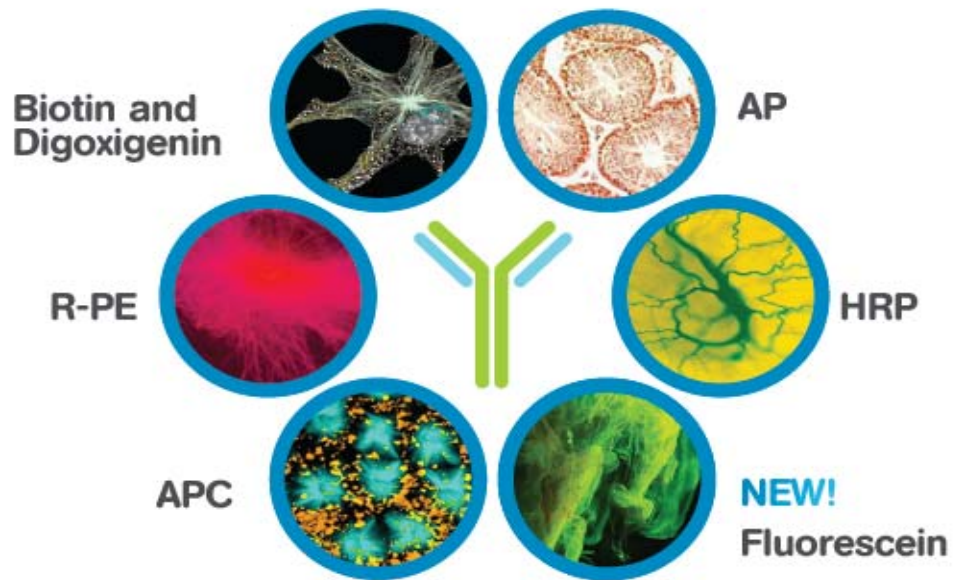
Streptavidin Agarose Ultra Performance™



 **3. Antibody Labeling Kits**

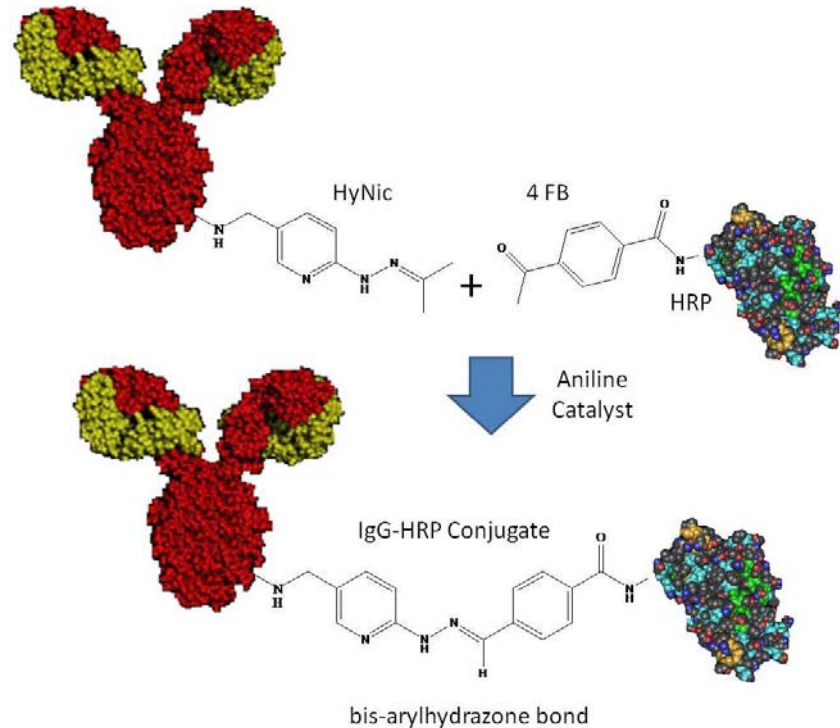
Antibody labeling

- The most efficient antibody labeling kits, delivering 100% purified and ready-to-use labeled antibodies
- All-in-One kits include purification component
- Applications
 - Flow cytometry
 - Western blotting
 - IHC



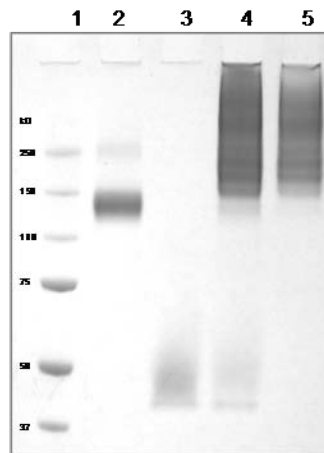
Labeling with HRP

- Researcher adds HyNic to the Ab
- HRP kit comes with 4FB-labeled HRP (ALREADY modified)
- Conjugate is traceable with the hydrazone bond



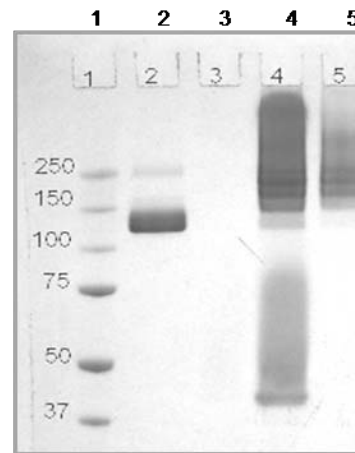
HRP-Antibody Conjugates (without Chromatography) Using Solulink All-in-One Kits

Bovine IgG-HRP Conjugate



Panel A

Mouse Anti-FITC-HRP Conjugate

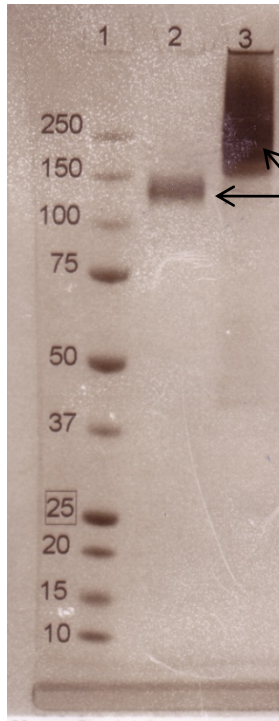


Panel B

**Poly HRP:
Higher signal**

1. Protein MW marker
2. IgG
3. 4FB-HRP
4. HyNic-Antibody + 4FB-HRP purified- crude
5. HyNic-Antibody + 4FB-HRP purified- purified

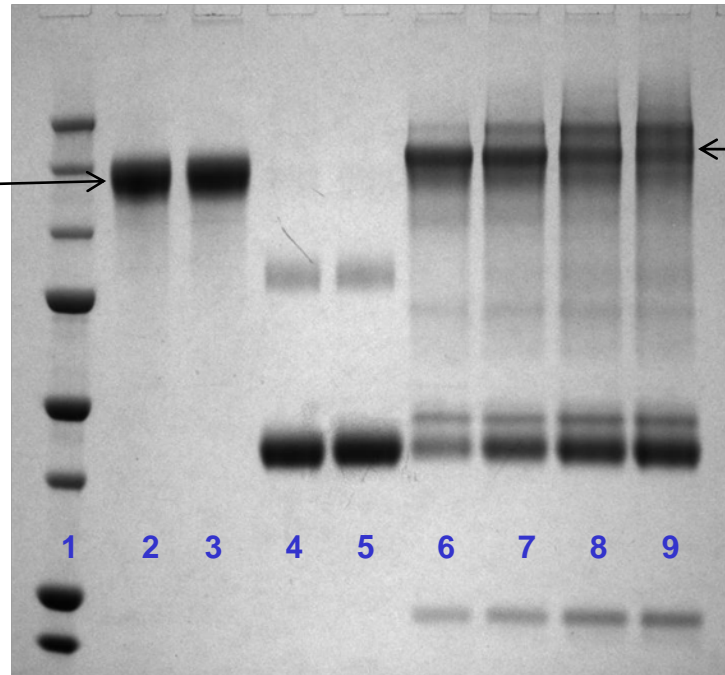
Solulink HRP/antibody conjugation



Note: Poly-HRP antibody conjugate is the preferred construct for optimal signal generation.

- 1) Protein ladder
- 2) HyNic-IgG
- 3) HyNic-IgG + 4FB-HRP (1/1; mol/mol)

Lightning Link HRP/antibody conjugation



- 1) Protein ladder
- 2) IgG
- 3) IgG-SH
- 4) HRP
- 5) HRP-mal
- 6) IgG-SH + HRP-mal (1/1; mol/mol)
- 7) IgG-SH + HRP-mal (1/2)
- 8) IgG-SH + HRP-mal (1/3)
- 9) IgG-SH + HRP-mal (1/4)

Top-Selling Antibody Labeling Products

(other than ChromaLink)

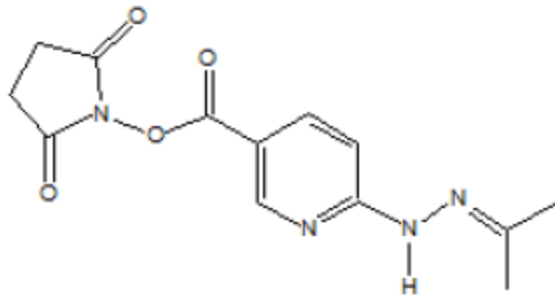
HRP-Antibody All-in-One Conjugation Kit

R-PE Antibody All-in-One Conjugation Kit



4. Crosslinking Kits and Reagents

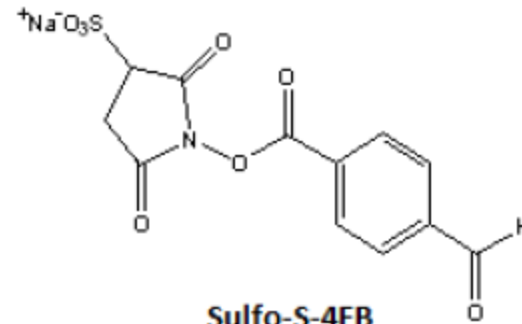
Solulink's 2 Crosslinkers (and variants of these)



S-HyNic

(C₁₃H₁₄N₄NaO₄; M.W.: 290.2)

S-HyNic (Succinimidly-6-hydrazino-nicotinamide)



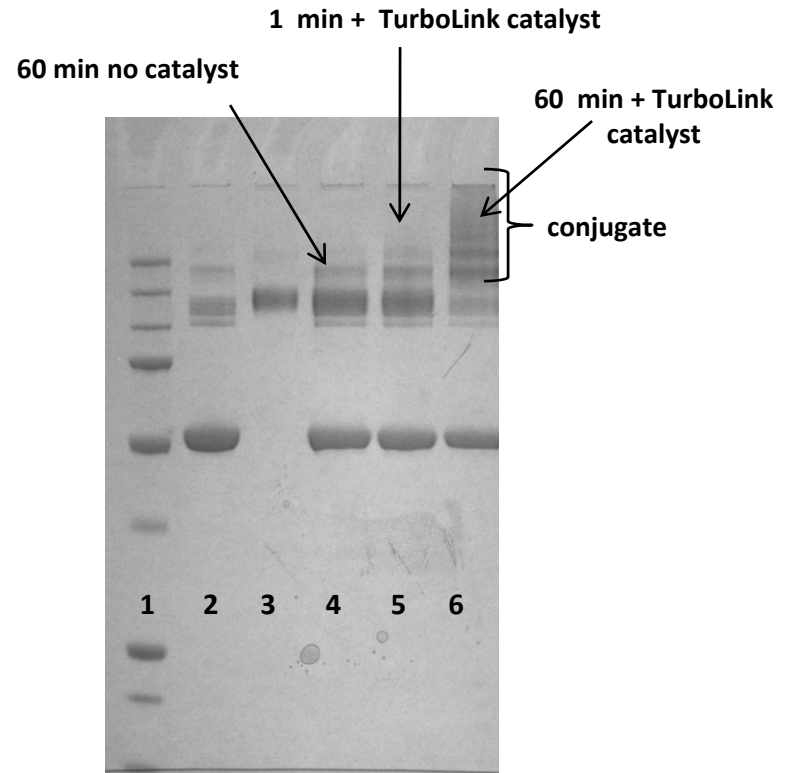
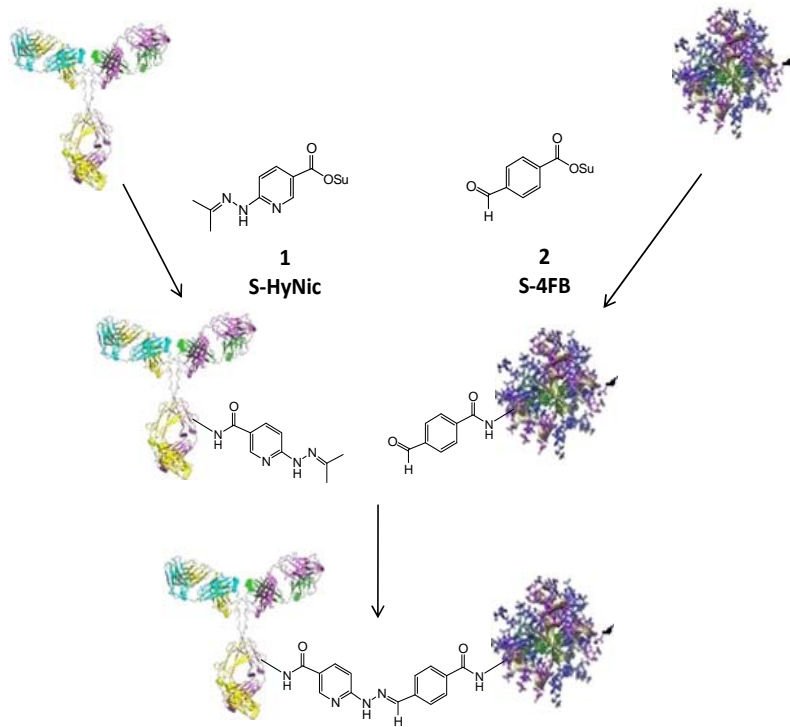
Sulfo-S-4FB

(C₁₂H₈NO₈SNa⁺; M.W.: 349.25)

Sulfo-S-4FB (Sulfo-N-succinimidly-4-formylbenzamide)

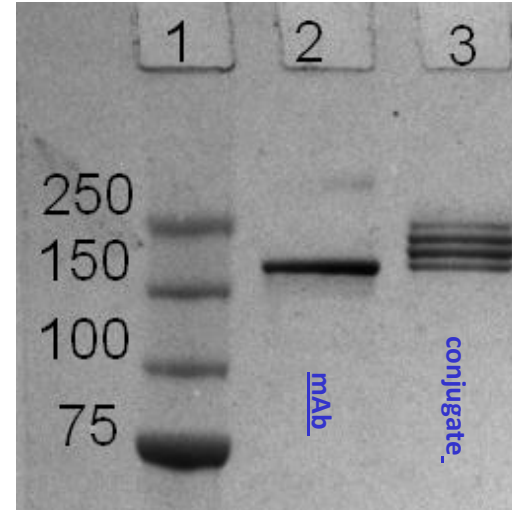
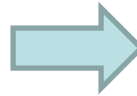
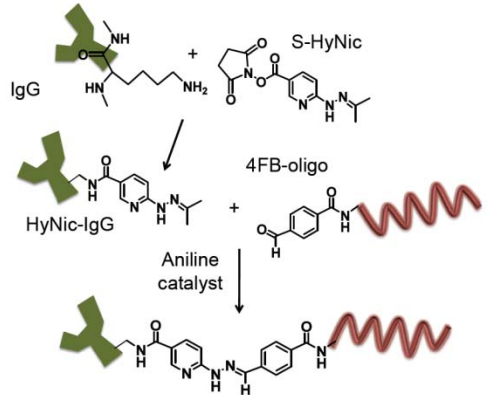
Protein-Protein Conjugation

Unprecedented protein-protein conjugation efficiency

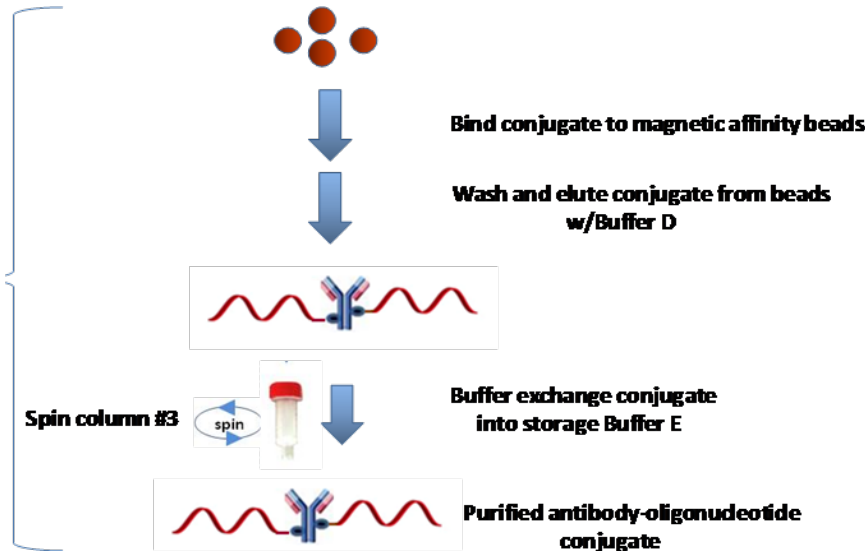


1. Ladder
2. 4FB-BSA
3. HyNic-IgG
4. 60 min no catalyst
5. 1 min catalyst
6. 60 min catalyst

Antibody-Oligo Conjugation



**>95% conversion to conjugate
NO HPLC required**



Top-Selling Crosslinking Kits and Reagents

Protein-Protein Conjugation Kit

Crosslinking reagents (S-HyNic and S-4FB)

Antibody-Oligonucleotide All-in-One Conjugation Kit



solulink™



5. Western Blot Kits

GET BETTER RESULTS.
GUARANTEED.



32
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RapidDirect Western Blot Kits

No Secondary Antibodies Necessary

Solulink's technology conjugates any primary antibody to polyHRP and offers several advantages over traditional secondary antibody detection methods:

- Publication-quality gels the first time
- Far less background—does not detect any antibody contaminants on blot, such as light and heavy chains
- A primary antibody conjugate is used
- Sensitivity better or equal to the classical secondary antibody methods in ALL immunoassay formats
- Significant time savings of 1.5 hours per blot or IHC tissue staining

RapidDirect Primary Antibody polyHRP Western Blot Kit
RapidDirect Primary Antibody polyHRP IP/Western Blot Kits

(with goat anti-mouse antibody)

(with goat anti-rabbit antibody)

(with rabbit anti-goat antibody)

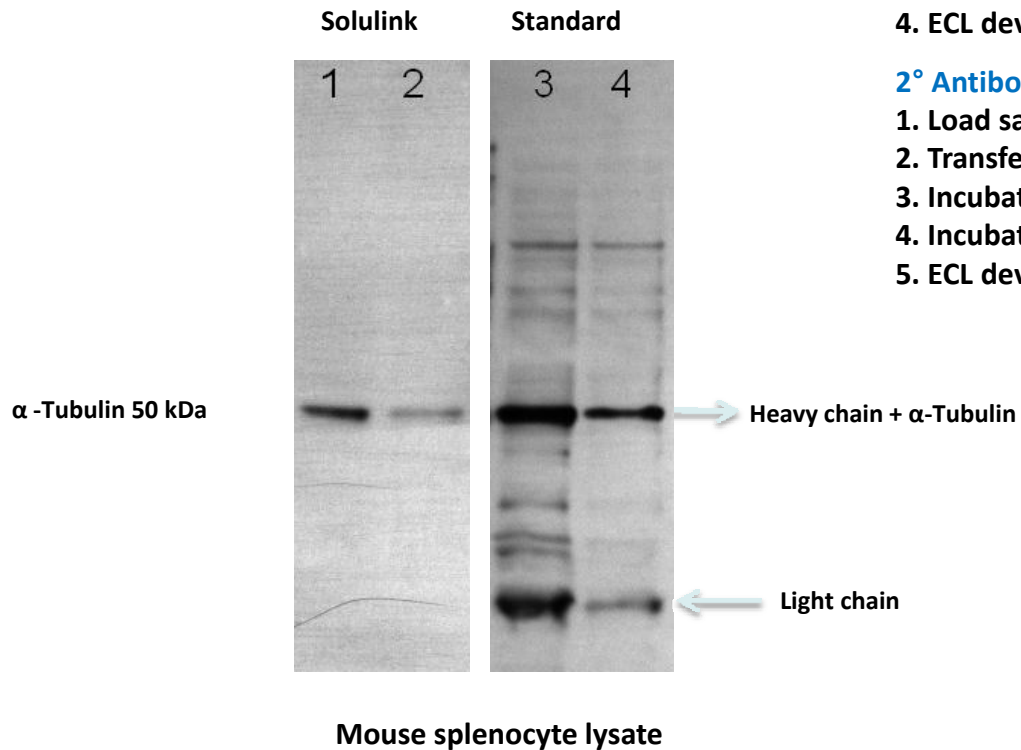
Direct Labeling of Primary Antibody for Western Blot Detection

Solulink Direct IP/Western Blot (Lane 1, 2)

1. Load sample 5 μ g to 2.5 μ g.
2. Transfer to nitrocellulose membrane, 3% milk block, 1 hour.
3. Incubate with 0.2 μ g/mL α -Tubulin antibody-HRP conjugate, 1 hour.
4. ECL development.

2° Antibody Western Blot Protocol (Lane 3, 4)

1. Load sample 5 μ g to 2.5 μ g.
2. Transfer to nitrocellulose membrane, 3% milk block, 1 hour.
3. Incubate with 0.2 μ g/mL α -Tubulin antibody, 1 hour.
4. Incubate with 0.15 μ g/mL goat anti-mouse-HRP conjugate, 1 hour.
5. ECL development





6. Custom Conjugation Services



Conjugation Services

Solulink can make the conjugates that others cannot.

- Our chemistry enables us to prepare conjugates that were previously very difficult or impossible to make.
- We can make custom conjugations to **ANY** antibody, oligo, protein, peptide, or solid surface.
- Bulk packaging or custom packaging is available.

Thank You!

Mike Hudson, Ph.D.

**Technical Services Manager &
International Distributor Manager**

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