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HRP-ANTIBODY ALL-IN-ONE™ CONJUGATION KIT

SKU: A-9002-001

DESCRIPTION

The HRP-antibody All-in-One Conjugation Kit contains everything needed to make two HRP-antibody conjugates from 100 µg of any user-supplied antibody. Included in the kit are purification columns to remove unconjugated HRP and antibody, which produces conjugates with superior levels of detection and low non-specific binding.

SPECIFICATIONS

Reactivity	Amine
Storage Instructions	2° – 8°C – Do Not Freeze
Applications	In Situ Proximity Ligation
Conjugate	Antibody
Label	HRP

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KIT COMPONENTS

- S-HyNic (2 x 100 µg)
- 4FB-HRP (2 x 100 µl)
- 1X Modification Buffer (10 mL)
- Q Binding Buffer A (5 mL)
- Q Elution Buffer A (1.6 mL)
- Red Cap Spin Column (2)
- Yellow Cap Spin Column B (2)
- Brown Cap Spin Column B (2)
- Blue Cap Spin Column B (2)
- Q Spin Column (2)
- Q Collection Tube (4)
- Anhydrous DMF (1.5 mL)
- 30K MWCO VivaSpin® Filter (2)
- 2 mL Collection Tube (16)

TECHNICAL INFORMATION

A. Product Description

Each HRP-antibody All-in-One Conjugation Kit provides all the necessary components to generate two (2) highly purified antibody-HRP conjugates. The kit requires the user to provide 100 µg of starting antibody for each conjugate. The components of this unique kit feature a pre-activated, high-activity horseradish peroxidase (>250U/mg) as well as a novel Q spin filter to purify the conjugate in high yield. Conjugates produced are free of both residual antibody and HRP, thus providing maximum signal to noise ratio in your assay. Any suitably purified monoclonal or polyclonal mammalian antibody (regardless of IgG subclass) can be conjugated and purified within 5 hours (~1 hour hands-on).

All-in-One conjugation kits are based on the SoluLINK bioconjugation technology. This chemistry involves the reaction of an aromatic hydrazine with an aromatic aldehyde to form a stable hydrazone bond. This conjugation is so efficient that it converts 100% of the antibody to the conjugate form. This linking efficiency is made possible because of the recent discovery that small quantities of aniline catalyze hydrazone bond formation between the two functional groups (1, 2, 3). Aniline increases both the rate and efficiency of conjugate formation under mild reaction conditions, leading to quantitative conversion of free antibody to HRP conjugate.

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Complete conversion of antibody to conjugate greatly simplifies downstream purification. Purification consists of selectively binding the conjugate to a novel Q spin filter membrane that allows excess HRP to flow through unbound. The spin filter provides high purity without sacrificing conjugate yield. Conjugates made with All-in-One kit are compatible with all downstream applications such as western blots, ELISAs, or IHC. Each kit provides sufficient reagents to perform two (2) conjugation reactions; each yielding between 50-70 µg of high purity HRP-antibody conjugate.

B. All-in-One Technology

Conjugation Chemistry

The SoluLINK bioconjugation technology is based on the use of two complementary heterobifunctional linkers; **S-HyNic** and **Sulfo-S-4FB** (Figure 1). **S-HyNic** (Succinimidyl-6-hydrazino-nicotinamide) is first used to modify and incorporate protected aromatic hydrazines (HyNic groups) into the antibody via acylation of lysine residues. In a similar fashion a second linker, **Sulfo-S-4FB** (Sulfo-N-succinimidyl-4-formylbenzamide) is used to provide a pre-activated high activity HRP called 4FB-HRP (included). Incubation of HyNic-modified antibody with pre-activated 4FB-HRP in the presence of aniline catalyst leads to rapid and efficient conversion of the antibody to conjugate through formation of stable bis-aryl hydrazone bonds (Figure 2).

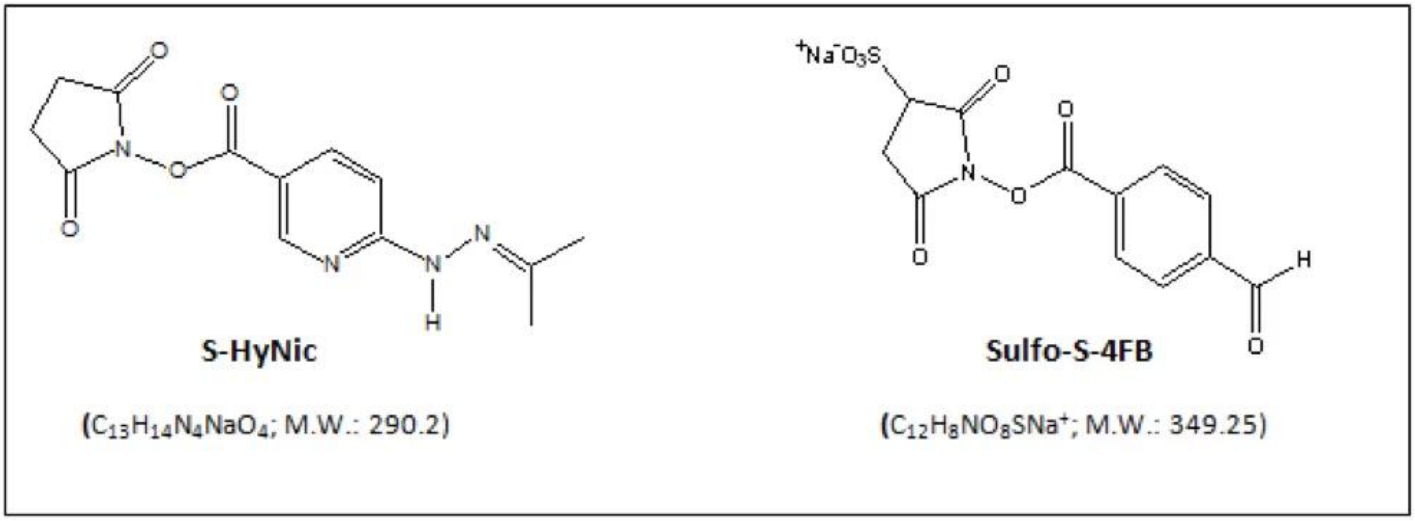

Figure 1. *Structure of S-HyNic and Sulfo-S-4FB linkers used for conjugating HRP to antibody.*

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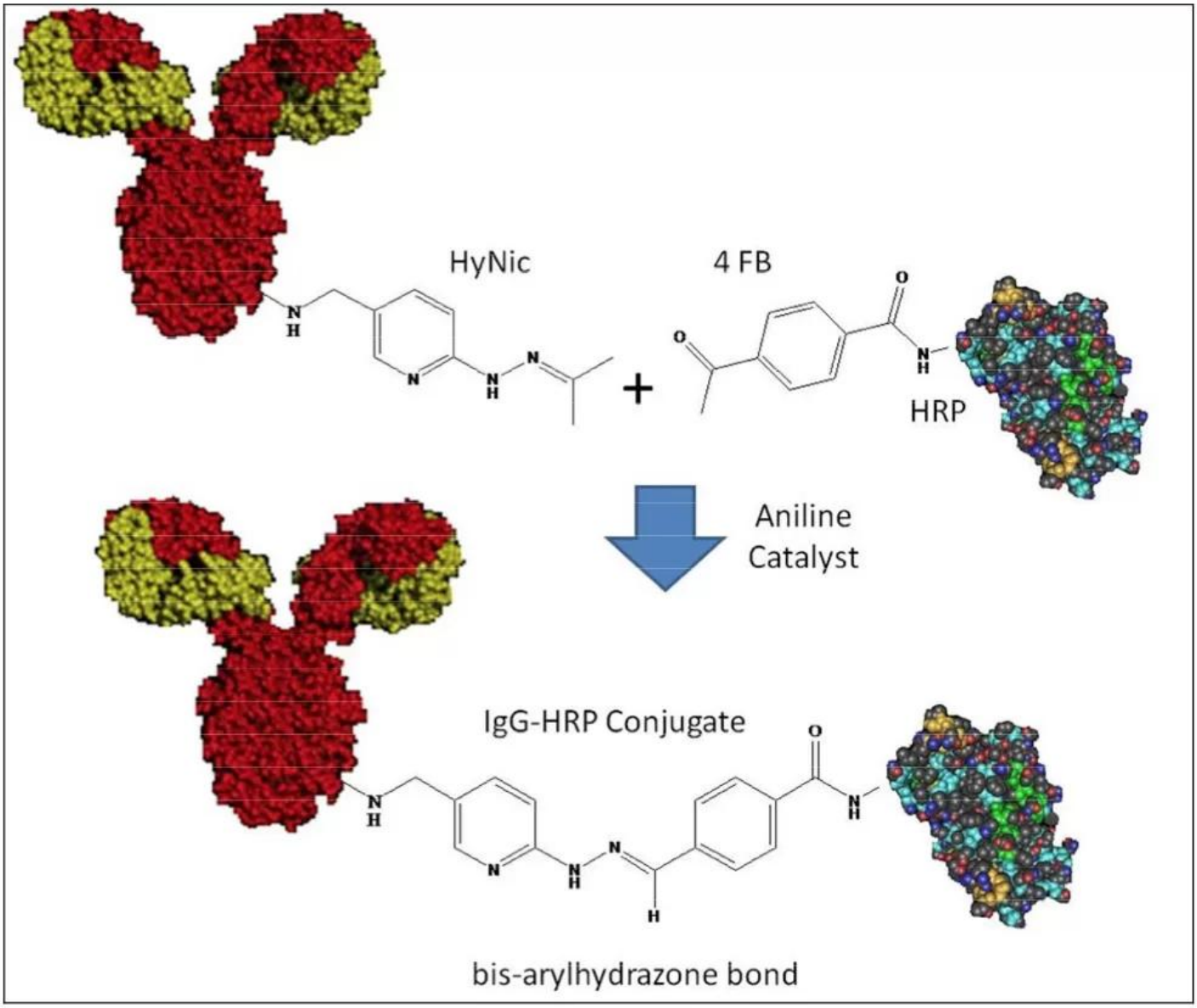

Figure 2. *Aniline catalyzed conjugation of HyNic-modified antibody with pre-activated 4FB-HRP.*

Conjugate Purification

The efficiency of aniline-catalyzed hydrazone bond formation greatly simplifies conjugate purification. Aniline's ability to increase both the rate and efficiency of conjugate formation under mild reaction conditions leads to quantitative conversion of free antibody to conjugate. The complete absence of free antibody at the end of the catalyzed reaction leaves only two components; excess HRP and conjugate. After conjugation, a novel Q spin filter is used to selectively bind the conjugate based on known biophysical properties of IgG (4, 5) while allowing free HRP to flow through. Purified conjugate can then be eluted from the filter

membrane free of both residual antibody and HRP in high yield (50-70 µg).

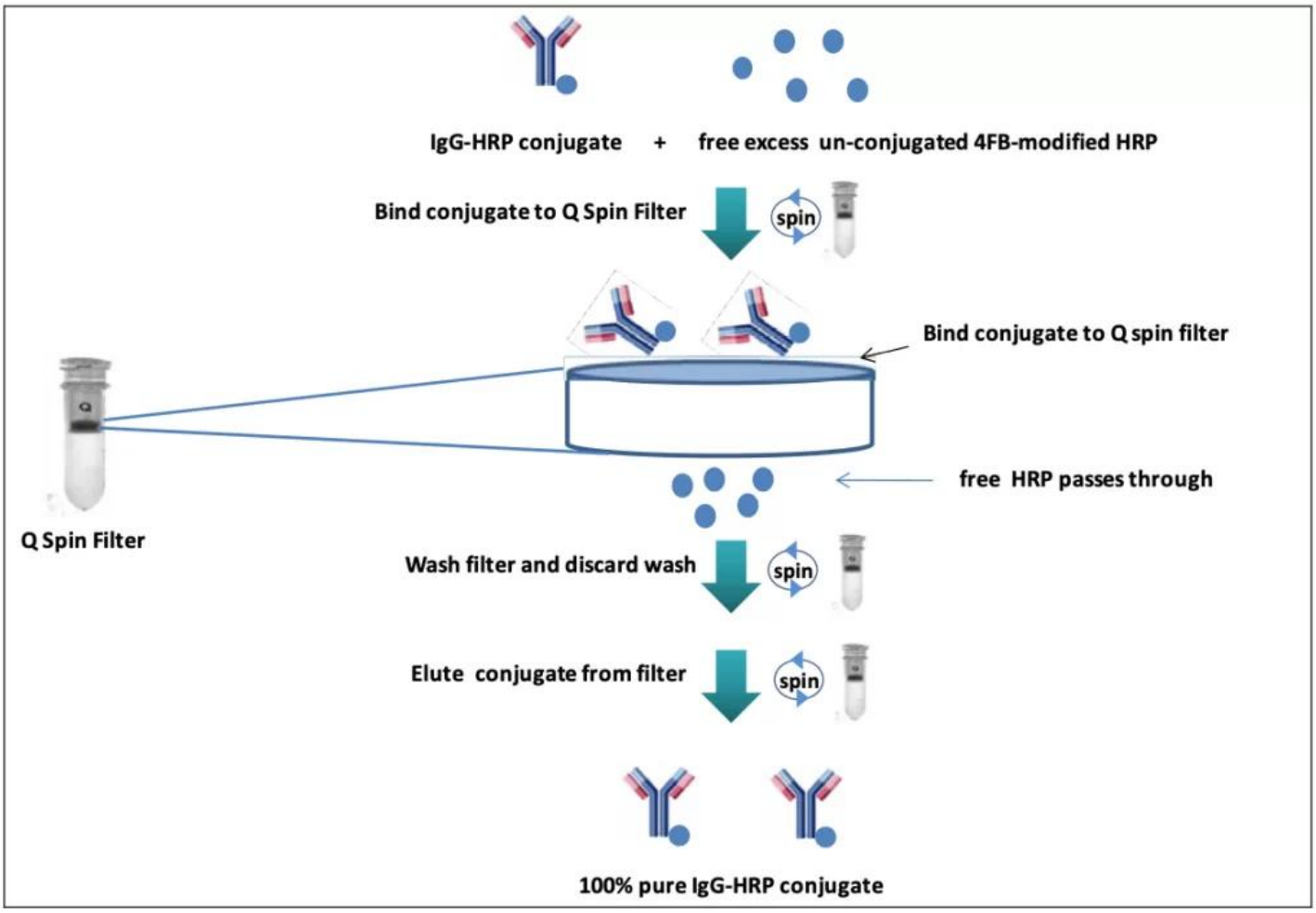

***Figure 3**. Q spin filter purification of HRP-IgG conjugate.*

C. All-in-One Conjugation Process Summary

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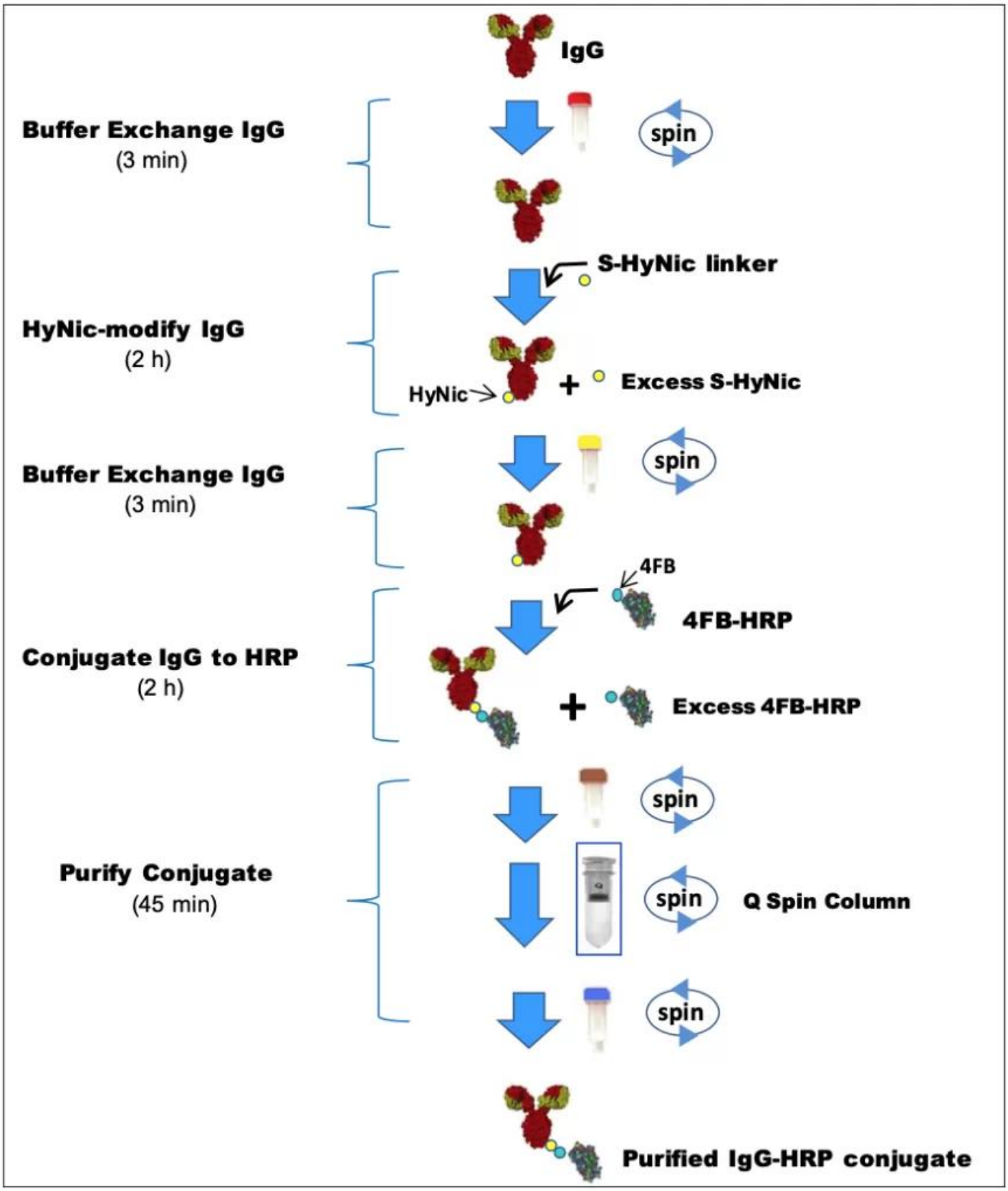

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Figure 4. *HRP-Antibody All-in-One conjugation process.*

CITATIONS

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DOCUMENTS

- User Guide
- Bioconjugation White Paper
- Safety Data Sheet
- Troubleshooting Guide – Bioconjugation
- Concentration of Dilute Antibody Solutions
- Using a NanoDrop to Measure Antibody Concentration
- BCA Protein Assay Protocol
- Bradford Assay Protocol
- Download CoA
- Datasheet

GALLERY IMAGES

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