

# HRP-Antibody All-In-One™ Conjugation Kit

**Cat. No.** A-9002-001

**Storage** 2°–8°C—Do Not Freeze.

The HRP-Antibody All-In-One Conjugation Kit requires 100 µg antibody at a concentration of 4 mg/ml in a total volume of 25 µl. The antibody buffer should be free of carrier proteins such as BSA or gelatin.

**Description** The HRP-Antibody All-In-One Conjugation Kit contains all the necessary reagents and components to produce two HRP-antibody conjugates. Based on **SoluLINK® bioconjugation technology**, it allows any purified antibody (free of carrier protein) to be conjugated and purified within 5 h, involving just 1 h of hands-on time. First, the user-supplied antibody is modified with S-HyNic, then the HyNic is conjugated to pre-activated 4FB-HRP. Next, a rapid spin filter is used to remove any residual antibody or HRP, resulting in 50–70 µg of high purity HRP-antibody conjugate that is ready for use (Figure 1).

## Kit Components

| Component                 | Amount     |
|---------------------------|------------|
| 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         |

## Protocol

Before using the HRP-Antibody All-In-One Conjugation Kit, remove from refrigerated storage and allow components to warm up to room temperature for at least 30 min.

### A. IgG Sample Preparation (10 minutes)

Use 1x Modification Buffer to dissolve lyophilized antibody or dilute aqueous antibody to a concentration of 4 mg/ml. If aqueous antibody is at less than 4 mg/ml, it must be concentrated. For guidance on checking the concentration of your antibody or concentrating a dilute antibody solution, please refer to the [Application Notes](#).

### B. Buffer Exchange IgG (3 min)

1. Remove one red cap spin column from the kit. Using a lab marker, place a vertical line on the side of the column.

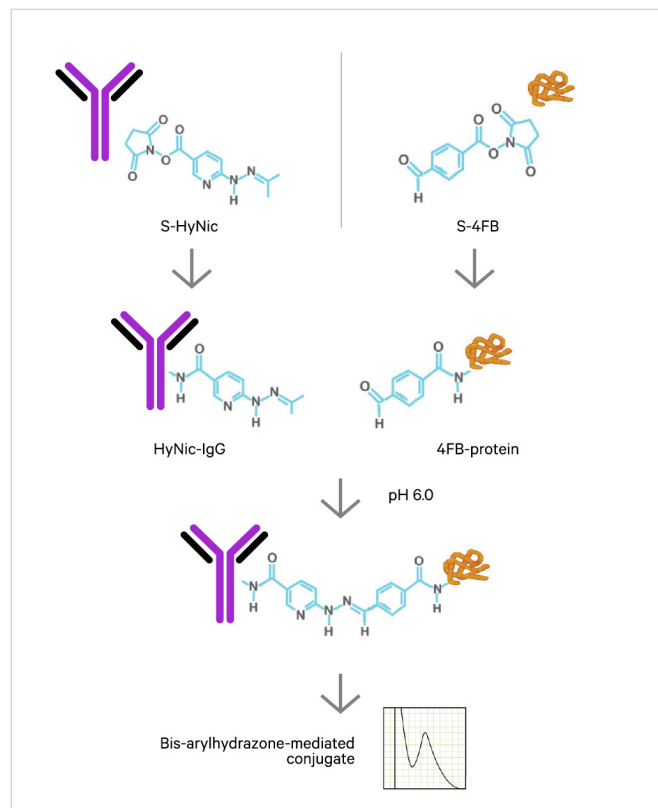


Figure 1. SoluLINK bioconjugation workflow.

2. Loosen the cap one-half turn, then twist and snap off the bottom closure. Place the spin column into a 2 ml collection tube (provided).
  3. Place the assembly into a centrifuge and orient the vertical mark on the spin column facing outward and away from the center of the rotor. Use an appropriate balance tube opposite the assembly.
  4. Centrifuge the column at 1,500 x g for 1 min (ensure the centrifuge is set to RCF or g and not RPM). Discard the collection tube containing storage buffer. The column matrix will appear white in color. Place the column into a new, empty collection tube (provided).
- Note:** Rotor speed must be set to 1,500 x g (RCF) and not 1,500 x rpm (RPM). The volume recovered should be approximately the same volume loaded onto the spin column (e.g., 25 + 5 µl). If the recovered volume is low, the centrifuge may require recalibration. If recovered volume is low, re-centrifuge at the appropriate speed to recover the full volume (i.e., 25 µl).
5. Remove the cap and load the antibody sample (25 µl at 4 mg/ml) to the top of the dry resin bed. Replace the cap and loosen one-half turn, then place the column back into the collection tube.
  6. Orient the spin column in the centrifuge with the mark facing outward as before and centrifuge at 1,500 x g for 2 min.

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7. Transfer the buffer-exchanged IgG solution (approximately 25  $\mu$ l) from the bottom of the collection tube to a new 1.5 ml microcentrifuge tube and label appropriately.

#### C. HyNic Modify IgG (2 h)

1. Add 20  $\mu$ l anhydrous DMF to the vial of S-HyNic reagent. Pipet the solution up and down several times to resuspend the reagent pellet. The HyNic pellet is very small but should be visible at the bottom of the vial.
2. Add 1.5  $\mu$ l of dissolved S-HyNic reagent to the antibody solution (25  $\mu$ l at 4 mg/ml). Briefly pipet the solution up and down, then gently vortex to thoroughly mix.
3. Incubate the reaction for 2 h at room temperature.

#### D. Buffer Exchange HyNic-Modified IgG (3 min)

1. 5 min before the end of the 2 h HyNic modification reaction prepare a yellow cap spin column.
2. Place a vertical line on the side of the column using a lab marker, loosen the cap one-half turn, twist/snap off the bottom closure, and place the column into a 2 ml collection tube.
3. Place the assembly into the centrifuge and orient the vertical mark on the spin column aiming outward and away from the center of the rotor. Use an appropriate balance tube opposite the assembly.
4. Centrifuge at 1,500 x g for 1 min (ensure the centrifuge is set to RCF or g and not RPM). Discard the collection tube containing storage buffer. The column matrix will appear white in color. Place the column into a new, empty collection tube.
5. Open the cap and load the now completed antibody-HyNic modification reaction (~25–35  $\mu$ l) to the top of the dry resin bed. Loosely cap the column and place back into the collection tube.
6. Orient the spin column in the centrifuge with the mark facing outward. Centrifuge at 1,500 x g for 2 min.
7. After centrifugation, transfer the solution (~25–35  $\mu$ l) from the bottom of the collection tube to a new 1.5 ml microcentrifuge tube. Label the sample appropriately (e.g., HyNic-IgG).

#### E. Conjugate Formation (2 h)

1. Briefly spin the dark brown vial containing 4FB-modified HRP to collect the contents at the bottom of the tube.
2. Transfer 50  $\mu$ l of 4FB-modified HRP to the tube containing HyNic-modified antibody and pipet up and down to mix.
3. Incubate the reaction for 2 h at room temperature to form the conjugate.

#### F. Buffer Exchange Conjugate (3 min)

1. After completion of the conjugation reaction, prepare a brown cap spin column.
2. Place a vertical line on the side of the column using a lab marker, loosen the cap one-half turn, twist/snap off the bottom closure, and place the column into a 2 ml collection tube.
3. Place the assembly into the centrifuge and orient the vertical mark on the spin column facing outward and away from the center of the rotor. Use an appropriate balance tube opposite the assembly.
4. Centrifuge at 1,500 x g for 1 min (ensure the centrifuge is set to RCF or g and not RPM). Discard the collection tube containing flow through. The column matrix will appear white in color. Place the column into a new, empty collection tube.
5. Open the cap and load the entire conjugate solution to the top of the dry resin bed. Loosely cap and place the column back into the collection tube.
6. Orient the spin column in the centrifuge with the mark facing outward and centrifuge at 1,500 x g for 2 min. Leave the solution in the collection tube.
7. After centrifugation, add 350  $\mu$ l of Q Binding Buffer A to the conjugate solution in the bottom of the collection tube and pipet up and down several times to mix. Set the solution aside on the bench while preparing the Q spin column.

#### G. Q Spin Column Purification (40 min)

1. Pre-wet a Q spin column by adding 200  $\mu$ l of Q Binding Buffer A to the top of the filter unit and incubating for 2 min.
2. Place the filter assembly into the centrifuge and orient the letter “Q” facing toward the center of the rotor. Place a balance tube opposite the Q column and spin at 2,000 x g for 4 min (ensure the centrifuge is set to RCF or g and not RPM). Discard the flow-through from collection tube and place the filter back into the empty collection tube.
3. Load the antibody-HRP conjugate (~430 – 450  $\mu$ l) from the previous section to the top of the filter unit and allow it to incubate on the filter for 2 min on the bench top.
4. Place the assembly in the centrifuge with the letter “Q” facing toward the center of the rotor and spin at 2,000 x g for 4 min with an appropriate balance tube opposite the filter unit.
5. Remove the flow-through from the bottom collection tube and place it in a microcentrifuge tube labeled “flow-through.” Place the filter back into the empty collection tube.

**Note:** A light brown color will appear on the top of the Q filter membrane; this is bound antibody-HRP conjugate. The flow-through may also contain a brown color (excess HRP). The flow-through fraction may be analyzed later in the unlikely event of low conjugate recovery.

6. Add 400  $\mu$ l of Q Binding Buffer A to the top filter unit, orient the letter "Q" facing toward the center of the rotor, and balance in the centrifuge. Spin at 2,000 x g for 4 min. Discard the flow-through from the bottom collection tube and place the filter back into the empty collection tube.
7. Repeat step 6 two additional times to completely remove unconjugated HRP.
8. Remove the top filter unit from the collection tube and place it into a new collection tube (provided).
9. Add 100  $\mu$ l of Q Elution Buffer A to the top filter unit and incubate for 5 min on the benchtop.
10. Place the assembly into the centrifuge, orient the letter "Q" facing toward the center of the rotor, and spin at 2,000 x g for 4 min. The eluate containing antibody-HRP conjugate may be left in the collection tube.
11. Add an additional 50  $\mu$ l of Q Elution Buffer A to the top of the filter unit and spin the assembly for another 4 min at 2,000 x g in the same collection tube. A slightly brown-colored, purified conjugate solution (150  $\mu$ l total volume) will now be at the bottom of the collection tube. Set the collection tube containing the antibody-HRP conjugate aside on the bench while preparing the blue cap spin column.

#### **H. Buffer Exchange Conjugate (3 min)**

1. Prepare a blue cap spin column by placing a mark on the side of the column, loosening the cap one-half turn, and twisting off the bottom closure. Place the spin column into a collection tube (provided).
2. Place the assembly into the centrifuge and orient the vertical mark on the spin column facing outward and away from the center of the rotor. Use an appropriate balance tube opposite the column.
3. Centrifuge at 1,500 x g for 1 min (ensure the centrifuge is set to RCF or g and not RPM). Discard the collection tube containing storage buffer. The column matrix will appear white in color. Place the column into a new, empty collection tube (provided).
4. Remove the cap and slowly load the conjugate eluted from the Q spin filter (collection tube contents from section G above) to the top of the dry resin bed. Loosely recap the column and place the column back into the new collection tube.
5. Orient the spin column in the centrifuge with the mark facing outward

and centrifuge at 1,500 x g for 2 min.

6. After centrifugation, transfer the buffer-exchanged antibody-HRP conjugate from the bottom of the collection tube to a new sterile 1.5 ml microcentrifuge tube. Label the microcentrifuge tube appropriately (e.g., purified antibody-HRP conjugate).
7. Measure the final protein concentration of the purified conjugate using either a Bradford or BCA protein assay, please refer to the Application Notes.

#### **Stability**

The HRP-Antibody conjugate can be stored at 2-8°C in final conjugate buffer for up to 6 months or mixed with an equal volume of glycerol and stored at -20°C for up to one year.

#### **Application Notes**

[Using a NanoDrop™ to measure antibody concentration](#)

[Concentrating dilute antibody solutions](#)

[Performing a Bradford assay](#)

[Performing a BCA protein assay](#)

[Troubleshooting Guide](#)