ReadyLabel™ Antibody Labeling Kits Protocols

Introduction

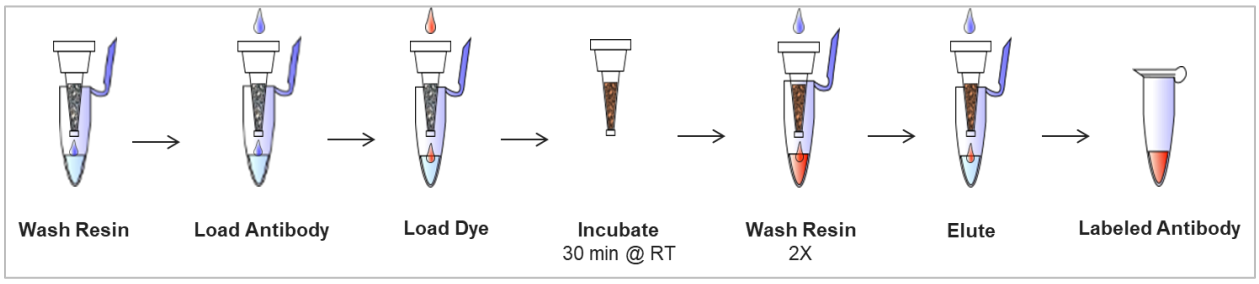
The Invitrogen™ ReadyLabel™ Antibody Labeling Kits allow for fast, convenient labeling of off-the-shelf antibodies and cell culture supernatants without prior purification of the antibody solution. Each ReadyLabel™ Antibody Labeling Kit contains everything needed to perform 5 labeling reactions and will produce purified antibody conjugates, without the purification step.

The kits can be used to label antibodies in solutions containing BSA or other stabilizing proteins, antibodies in a primary amine-containing buffer such as Tris or glycine, or even cell culture supernatants, without prior antibody purification. The reactive dye efficiently reacts with primary amines of antibodies to form stable, covalent conjugates. For smaller amounts of antibody, the ReadyLabel™ 20 µg Antibody Labeling Kit will provide superior results; for larger quantities use the ReadyLabel™ 100 µg Antibody Labeling Kit. ReadyLabel™ Flex kits are designed for use with a variety of amine-reactive dyes.

Materials

Included Materials	Additional Materials
ReadyLabel™ spin columns (Component A)	Antibody solution, or cell culture supernatant
ReadyLabel™ Wash Buffer (Component B)	Benchtop centrifuge capable of up to 1,000 x *g*
Dimethylsulfoxide (DMSO) (Component C)	1.5 or 2 mL microcentrifuge tubes
ReadyLabel™ Labeling Buffer (Component D)	Pipettors and disposable pipette tips
ReadyLabel™ Neutralization Buffer (Component E)	
ReadyLabel™ Elution Buffer pH 3.3 (Component F)	
ReadyLabel™ Elution Buffer pH 2.0 (Component G)	
Reactive Dyes	

Graphical Protocol



Procedure for 100 µg ReadyLabel™ kits

Prepare the spin column

1. Twist to remove the bottom plug of the column (**Component A**). Loosen the cap but do not fully remove.
2. Place the column in a collection tube, then centrifuge the column-tube assembly at 1,000 x *g* for 30 seconds to remove the storage buffer. Discard the flowthrough. The cap may be discarded after this step.
3. Add 500 µL of Wash Buffer (**Component B**) to the column, then centrifuge at 1,000 x *g* for 30 seconds. Discard the flowthrough.

Protocol Tips

- Do not reuse the spin columns.
- This kit is designed for IgG antibody labeling and cannot be used to label other proteins.
- Work quickly during the dye dissolution and loading steps; once dissolved the dye begins to lose reactivity, which could result in a reduced degree of labeling.

Purify and label the antibody

1. Load 100 µg of antibody solution onto the washed resin, then centrifuge at 100 x *g* for 2 minutes.
2. Additional spin time may be needed if residual liquid remains above the resin in the column. Do not proceed to the next step until all liquid has flowed through the resin.
3. Add 7 µL DMSO (**Component C**) to a vial of lyophilized dye and pipette gently to dissolve the dye.
4. Dilute the dissolved dye with 65 µL of Labeling Buffer (**Component D**).
5. Immediately add 60 µL of the diluted dye onto the resin and centrifuge at 100 x *g* for 1 minute. Discard the flowthrough.
6. Incubate at room temperature for 30 minutes.

Elute the labeled antibody

1. Add 500 µL Wash Buffer (**Component B**) to the column, then centrifuge at 1,000 x *g* for 30 seconds. Discard the flowthrough.
2. Repeat the wash step (Step 1).
3. Add 60 µL Neutralization Buffer (**Component E**) to a clean 1.5 mL centrifuge tube and transfer the washed column to this tube.
4. Add 240 µL Elution Buffer pH 3.3 (**Component F**) to the resin, then centrifuge at 300 x *g* for 3 minutes.
5. If a poor yield is achieved, Elution Buffer 2.0 (**Component G**) may be substituted for Elution Buffer 3.3, or they may be used sequentially.

ReadyLabel™ Flex Kit Recommendations

- ReadyLabel™ Flex kits are designed for use with a variety of amine-reactive dyes.
- Use 100 µg of dye per 100 µg antibody labeling reaction or 20 µg of dye per 20 µg antibody labeling reaction.
- Dissolve reactive dye with DMSO (**Component C**) using less than 1/10 of the final diluted volume. Then dilute to ~1 mg/mL in labeling buffer (**Component D**).
- For example, dissolve a 100 µg vial of lyophilized reactive dye with 10 µL DMSO, then dilute with 90 µL labeling buffer to a final volume of 100 µL at 1 µg/µL. Use for 1 x 100 µg or 5 x 20 µg reactions.

Procedure for 20 µg ReadyLabel™ kits

Prepare the spin column

1. Twist to remove the bottom plug of the column (**Component A**). Loosen the cap but do not fully remove.
2. Place the column in a collection tube, then centrifuge the column-tube assembly at 1,000 x *g* for 30 seconds to remove the storage buffer. Discard the flowthrough. The cap may be discarded after this step.
3. Add 100 µL of Wash Buffer (**Component B**) to the column, then centrifuge at 1,000 x *g* for 30 seconds. Discard the flowthrough.

Purify and label the antibody

1. Load 20 µg of antibody solution onto the washed resin, then centrifuge at 200 x *g* for 2 minutes.
2. Additional spin time may be needed if residual liquid remains above the resin in the column. Do not proceed to the next step until all liquid has flowed through the resin.
3. Add 2 µL DMSO (**Component C**) to a vial of lyophilized dye and pipette gently to dissolve the dye.
4. Dilute the dissolved dye with 18 µL of Labeling Buffer (**Component D**).
5. Immediately add 15 µL of the diluted dye onto the resin and centrifuge at 200 x *g* for 2 minutes. Discard the flowthrough.
6. Incubate at room temperature for 30 minutes.

Elute the labeled antibody

1. Add 100 µL Wash Buffer (**Component B**) to the column, then centrifuge at 1,000 x *g* for 30 seconds. Discard the flowthrough.
2. Repeat the wash step (Step 1).
3. Add 60 µL Neutralization Buffer (**Component E**) to a clean 1.5 mL centrifuge tube and transfer the washed column to this tube.
4. Add 240 µL Elution Buffer pH 3.3 (**Component F**) to the resin, then centrifuge at 300 x *g* for 3 minutes.
5. If a poor yield is achieved, Elution Buffer 2.0 (**Component G**) may be substituted for Elution Buffer 3.3, or they may be used sequentially.

Optimization and troubleshooting

Working with antibody solutions containing glycerol

- The following tables include suggested guidelines for loading antibodies containing varying amounts of glycerol. These times are a suggested starting point; additional spin time may be required.
- It may be necessary to increase the speed of centrifugation if the antibody/glycerol solution does not flow through the resin in a reasonable amount of time. Note that increased speeds may lead to reduced antibody yield.

20 µg ReadyLabel™ Glycerol Guidelines				
Percent glycerol	**Antibody loading spin time (minutes)**	**Spin speed (*xg*)**	**Dye loading time (minutes)**	**Spin speed (*xg*)**
0	2	200	2	200
10	4	200	4	200
25	6	200	4	200
50	8	200	4	200

100 µg ReadyLabel™ Glycerol Guidelines				
Percent glycerol	**Antibody loading spin time (minutes)**	**Spin speed (*xg*)**	**Dye loading time (minutes)**	**Spin speed (*xg*)**
0	2	100	2	200
10	4	100	2	200
25	6	100	3	200
50	6	100	3	200

Storage and handling of conjugates

- Store the labeled antibody conjugate at 2-8°C, protected from light.
- Eluted antibody conjugates will typically be between pH 7 and pH 8.5 and should be used within two weeks for best results.
- For longer storage, add 2 mM sodium azide or buffer exchange into a solution containing 2 mM sodium azide to prevent contamination.
- For longer storage of dilute (< 1 mg/mL) purified antibody conjugates, add BSA or other stabilizing protein at 1 – 10 mg/mL (0.1 – 1% BSA).
- For long-term storage, divide the conjugate into small aliquots and freeze at ≤ -20°C. Avoid repeated freezing and thawing.
- For optimal use, centrifuge solutions of conjugates in a microcentrifuge before use; only the supernatant should then be used in the experiment. This step will remove any aggregates that may have formed during storage.

Inefficient removal of free dye

Despite removing most free dye from antibody conjugates using the provided resin columns, it is possible that small amounts of free dye will be present in the conjugate solution. The presence of free dye, which can be determined by thin layer chromatography, will result in erroneously high calculated values for the degree of labeling. Remaining free dye can be removed by applying the conjugate to a Zeba™ Dye and Biotin Removal Spin Column (A44296) or by extensive dialysis. For typical applications that include a washing step after the antibody conjugate has been applied to cells or tissue, any residual dye will be removed by the wash step.

Inefficient removal of BSA or contaminating proteins

Despite removing most contaminating proteins from antibody conjugates using the provided resin columns, it is possible that small amounts of BSA or other protein will be present in the conjugate solution. The presence of contaminating proteins, which can be determined by SDS PAGE, will result in erroneously high calculated values for the concentration of the conjugate solution. For typical applications that involve a blocking step prior to application of the conjugate and a wash step after the application of the conjugate, contaminating protein will be removed by the wash step.

Catalog Number	Description
R10701	ReadyLabel™ Flex Antibody Labeling Kit for 5 X 20 µg of antibody
R10702	ReadyLabel™ Flex Antibody Labeling Kit for 5 X 100 µg of antibody
R10709	ReadyLabel™ AlexaFluor™ 488 Antibody Labeling Kit for 5 X 20 µg of antibody
R10710	ReadyLabel™ AlexaFluor™ 647 Antibody Labeling Kit for 5 X 20 µg of antibody
R10711	ReadyLabel™ Biotin Antibody Labeling Kit for 5 X 20 µg of antibody
R10712	ReadyLabel™ AlexaFluor™ 488 Antibody Labeling Kit for 5 X 100 µg of antibody
R10713	ReadyLabel™ AlexaFluor™ 647 Antibody Labeling Kit for 5 X 100 µg of antibody
R10714	ReadyLabel™ Biotin Antibody Labeling Kit for 5 X 100 µg of antibody