Omni® 647 SE Labeling Kit

Product Code：OM700023

Package Contents:

Composition：	OM700023 (5-20 μg)	OM700023 (20-50 μg)	OM700023 (50-100 μg)
A: Reactive dye vial 2–6℃ or ≤–20℃, if desired Protect from light Protect from moisture	1 vial	1 vial	1 vial
B: Sodium bicarbonate (MW = 84) 10 × ~84mg，Protect from moisture	84mg	84mg	84mg
C:Purification columns Store at room temperature	1column	1column	1column
D：Purification resin 2–6℃ only; DO NOT FREEZE	~5mL	~5mL	~5mL
E：Ultrafiltration vial MWCO=10K	1 each	1 each	1 each

Storage Conditions: All components should be stored at the temperature indicated on their labels.

Expiration date can be found on the outer packaging.

Product Introduction

Omni® Labeling Kit provides all necessary components for rapid and efficient labeling of antibodies using our proprietary dyes or biotin (Biotin). The labeling process is simple—just mix the antibody with the dye in the provided reaction buffer and incubate for a short time. After labelling, approximately 4-6 molecules of dye are covalently bound to 1 molecule of antibody. the Omni® Labeling Kit is covalently labelled so that antibodies labelled with it can be used for multicolour fluorescent staining without dye transfer between antibodies.

Omni® Labeling Kit is compatible with NaN3, low concentrations of glycerol, Tris, and glycine. The included mini ultrafiltration tubes can rapidly remove incompatible small-molecule antibody stabilizers prior to labeling.

The Omni® Labelling Kit is suitable for labelling proteins such as IgG. For labelling of other non-IgG proteins, the amount of dye used should be calculated according to the characteristics of the different proteins to determine the labelling rate (DOL). Note: This kit may cause denaturation of IgM antibodies.

Commercially labeled secondary antibodies can still recognize primary antibodies labeled with the Omni® kit. Therefore, when multiple antibodies from the same species are used for multiplex immunofluorescence staining, the secondary antibodies

cannot distinguish between unlabeled primary antibodies and those labeled with the Omni® kit. Primary antibodies labeled with the Omni® kit can also be used for tertiary staining following traditional indirect immunofluorescence detection.

Table 1. Omni® Antibody Compatibility and Labelling Procedure Selection Guide

Composition	compatibility
NaN_3	Compatible
Glycerol	≤10%： Standard Steps（StepB） >10%：Super filter（StepA）
Tirs	≤20 mM：standard steps（StepB） >20 mM：Super filter（StepA）
Glycine	Super filter（StepA）
BSA or gelatin	≤4times IgG μg：standard steps（StepB） >4times IgG μg：re-sample
Ascites	optimisation steps（StepC）
Serum	Not compatible：purified IgG
Hybridoma cell line culture supernatant	Not compatible：purified IgG

Preparation

1. check your antibody against the Antibody Compatibility Guide (Table 1): if your primary antibody is a commercial product, contact the supplier for antibody concentrations and buffer formulations. Omni® labelling kits cannot be used for labelling of natural antisera or culture supernatants of hybridoma cell lines, and antibody purification is required for labelling.

2. Antibody solutions without stabilisers give better labelling results, while the standard labelling procedure is compatible with low concentrations of glycerol and the labelling solution is not affected by NaN3. For the standard labelling procedure (B), select the kit size according to the amount of IgG (ug) to be labelled.

3. The Optimisation Step (C) of the Omni® Labelling Kit is designed to be based on the total amount of labelled protein rather than the amount of IgG labelled. The optimisation step should be used for labelling antibodies that contain too much stable protein. Antibody labelling in solution is also suitable for the optimisation step, but you must determine the concentration and total amount of total protein in the solution before labelling (protein concentration is estimated by measuring the absorbance at 280 nm). The optimisation step can also be used to label samples with IgG amounts below the minimum range of the kit, by adding stabilising proteins to bring the total amount of protein within the labelled range of the kit.

4. Labelled antibodies containing low concentrations of BSA and gelatin may have a slightly stronger staining background than antibodies without these stabilisers. If antibody labelling is performed in the presence of BSA or gelatin, the use of a blocking and cleaning agent containing 1% BSA or gelatin will greatly reduce the staining background (these should be avoided if possible). Non-protein components such as Tris, glycine or glycerol are removed and your antibody can be purified using the ultrafiltration tubes provided in the kit as follows (A).

5. Antibodies with high labelling efficiencies are available in concentrations of 1-5 mg/mL. Ultrafiltration tubes can be used to concentrate the antibody (note: stabilised proteins will also be concentrated in the ultrafiltration vial). To quantify an unknown concentration of antibody, follow the standard procedure (B) or the optimisation procedure (C) if you do not need to remove contaminants or concentrate the antibody.

A. Sample Handling

IMPORTANT: Before you begin, check Table 1 (Omni® Antibody Compatibility and Labelling Procedure Selection Guide) to determine if your antibody requires ultrafiltration prior to labelling. If so, contact the antibody manufacturer for protein stabiliser concentrations. If your antibody does not require ultrafiltration, select the appropriate labelling step according to Table 1. The ultrafiltration membrane has a molecular weight cutoff of 10 KD (this cutoff can be communicated in advance so that we can ship ultrafiltration tubing sized for your protein), so heteroproteins with molecular weights less than 10 kDa will pass through the membrane while proteins with molecules greater than 10 kDa, including IgG, will be retained on the membrane (Figure 1). Be careful not to touch the membrane with the pipette; this can tear or puncture the membrane and result in antibody loss. ***Proteins must have a minimum molecular weight of ≥20 KD to be ultrafiltered***.

Ultrafiltration Tube Performance

Maximum sample volume：500 µL　　Filtrate receiver volume：500 µL

Final concentration volume：15 µL　　Retention volume (membrane/support)：< 5 µL

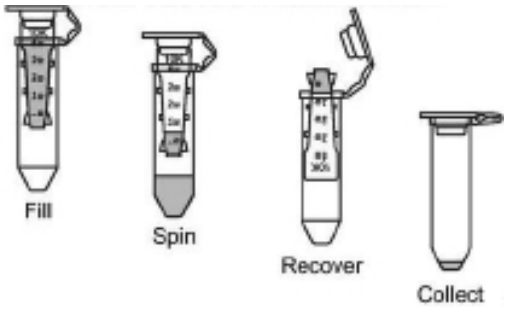


Figure 1. Ultrafiltration tube composition

1. Add the appropriate amount of antibody to the ultrafiltration tube, being careful not to touch the membrane.

Centrifuge at 8000 g for 1 min and check how much liquid has filtered into the filtrate collection tube (below). Repeat until all liquid is collected in the filtrate collection tube. Remove the liquid from the collection tube.

2. For concentrated antibodies, proceed directly to step 3. For purified antibodies, add an equal volume of 1 x PBS to the ultrafiltration tube. centrifuge at 8000 g until the liquid filters into the filtrate receiving tube.

3. Add an appropriate volume of PBS to the membrane of the ultrafiltration tube to achieve a final antibody concentration of 1-5 mg/mL, and resuspend the antibody by carefully aspirating up and down the membrane surface. 4. Transfer the recovered antibody solution to the membrane.

4. Transfer the recovered antibody solution to a new centrifuge tube. 5.

5. If you are using the Optimised Labelling Procedure, save the ultrafiltration tube to concentrate your labelled antibody.

B.Omni® Standard Labelling Procedure

IMPORTANT: Before you begin, compare Table 1 (Omni® Antibody Compatibility and Labelling Procedure Selection Guide) to determine if your antibody composition and concentration are suitable for Omni® labelling and which labelling procedure is appropriate for your choice. If necessary, contact the antibody manufacturer to find out the concentration of your antibody IgG and antibody stabiliser. The molecular weight of the marker must be known and above 20KD for this procedure.

1. Protein (IgG and stabiliser proteins) concentration 1-5 mg/mL is the appropriate concentration for labelling, dissolve or dilute with PBS if required. Ensure that the total μg of protein matches the labelling range of the kit. If the IgG to be labelled is less than the minimum labelling amount of the kit, add BSA so that the total protein amount of BSA and IgG is appropriate for the kit.

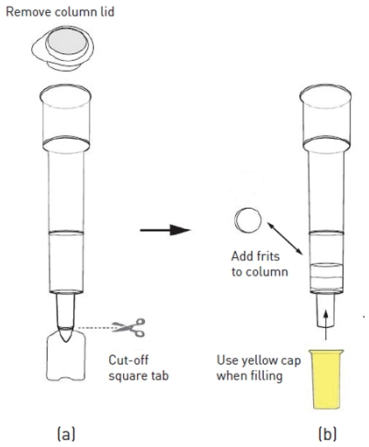
2. Dissolve component B in 1 mL of distilled water to make 10 × reaction buffer. 3.

3. Mix the 10 × reaction buffer solution with the antibody at 1:10 so that the antibody solution ends up as 1 × reaction buffer (e.g., 9 μL of antibody plus 1 μL of 10 × reaction buffer). Pipette up and down to mix.

4. Transfer all of the solution from the previous step to a Omni® dye vial. There is no need to check the level of dye in the vial here; vortex for a few seconds to dissolve the dye.

5. Incubate for 30 min at room temperature, preferably vortexing every 10 min, to allow the dye and protein to come into contact with each other.

6. Assemble the purification column during the reaction as shown in the figure. Mix the purification packing into the column chamber and release the protective solution until the volume of the column bed is around 1 mL.



7. Flow the reacted labelled conjugate through the purification column by gravity and collect the flow-through solution (roughly 40 µL per drop). Depending on the volume of conjugate you add, you can decide how much to collect. You can collect more flow-through solution for subsequent ultrafiltration.

8. If the molecular weight of the marker is above 20KD, you may choose to concentrate the conjugate using the previous ultrafiltration tube.

9. The determination of dye-protein conjugate concentration can be calculated by the following formula:

$$C(mg/mL) = \{[A280 - (Amax \times Cf)]/1.4\} \times n$$

a. C is the dye-protein conjugate concentration;

b. N is the number of times diluted during the photometric measurement;

c. A280 and Amax are the absorbance at 280 nm and at the absorption wavelength, respectively;

d. Cf is the correction factor;

Note: Protein solutions eluted from the column may be too concentrated to be used directly in the absorbance assay and therefore need to be diluted to approximately 0.1 mg/mL.The dilution factor needs to be predicted from the initial amount of antibody as well as the total volume of protein solution eluted.

10. Store the labelled protein at 2-8°C protected from light. If the final concentration of the purified protein conjugate is less than 1 mg/mL, add an appropriate amount of BSA or other stabilising protein. The conjugate should be stable for several months at 2-8°C in the presence of 2 mM sodium azide. For longer storage, freeze the conjugate in small aliquots at ≤ -20°C. Avoid repeated freezing and thawing and store away from light.

C. Omni® Optimised Labelling Procedure

IMPORTANT: Before you begin, compare Table 1 (Omni® Antibody Compatibility and Labelling Procedure Selection Guide) to determine if your antibody composition and concentration are suitable for the Omni® Labelling Kit and which labelling procedure is right for you. If necessary, contact the antibody manufacturer to find out the concentration of your antibody/IgG stabiliser.

1. Antibody Concentration 1-5 mg/mL is the appropriate labelling concentration. If the antibody is in lyophilised powder form or at a more concentrated concentration, dissolve or dilute the antibody in PBS. Transfer the antibody to be labelled into a clean EP tube. Ensure that the µg number of IgG matches the labelling range of the kit.

2. Dissolve Component B well in 1 mL of distilled water to make 10 × reaction buffer. 3.

3. Mix the 10 × reaction buffer solution with the antibody at 1:10 so that the antibody solution ends up as 1 × reaction buffer (e.g., 9 µL of antibody plus 1 µL of 10 × reaction buffer). Blow up and down the mixture with a pipette.

4. Transfer all of the solution from step 3 to a Omni® dye vial, where there is no need to test the vial for dye content, and vortex for a few seconds to dissolve the dye.

5. Incubate for 30 min at room temperature away from light, preferably vortexing and mixing every 10 min.

6. Clean the ultrafiltration tube during the reaction. Transfer the labelled protein solution into the processed ultrafiltration tube, centrifuge at 8000g for about 10 minutes, discard the filtrate, add 1XPBS to the cartridge and repeat centrifugation at least four times to obtain the pure dye-antibody conjugate.

7. Add buffer to the ultrafiltered conjugate to meet your needs for subsequent experiments.

8. The concentration of the dye-protein conjugate can be determined by the following formula:

$$C(mg/mL) = \{[A280 - (Amax \times Cf)]/1.4\} \times n$$

a. C is the dye-protein conjugate concentration;

b. N is the number of times diluted during the photometric measurement;

c. A280 and Amax are the absorbance at 280 nm and at the absorption wavelength, respectively;

d. Cf is the correction factor;

9. Store the labelled protein at 2-8°C, protected from light. If the final concentration of the purified protein conjugate is less than 1 mg/mL, add an appropriate amount of BSA or other stabilising protein. The conjugate should be stable for several months at 2-8°C in the presence of 2 mM sodium azide. For longer storage, divide the conjugate into small aliquots and store frozen at ≤ -20°C. Avoid repeated freezing and thawing during this period and store away from light.

Remarks: We can provide different purification methods and materials according to customers' experimental needs, which need to be informed by customers in advance.