User Guide

SMC® Capture Labeling Kit Instructions

Microparticle Assay

Kit instructions for the labeling of capture antibody and conjugation to microparticles for use with SMC® Analyte Specific Immunoassays

03-0077-02

For research use only. Not for use in diagnostic procedures.

Introduction

The SMC® Immunoassay System uses a quantitative fluorescent sandwich immunoassay technique to measure analyte in matrix. This kit is intended for the labeling of 0.3-1.0 mg of a capture antibody (Ab) and conjugation to magnetic microparticle (MP) beads. The labeled capture antibody is used in conjunction with a sandwich immunoassay to be developed for the SMC® Immunoassay System.

This kit should be used in conjunction with (03-0076-02) SMC® Detection Labeling Kit and (03-0078-00) SMC® Bead Based Immunoassay Development Kit.

Antibodies used are to be in carrier- and label-free buffer.

Note: If labeling proteins other than antibodies of a 150 kD approximate molecular weight, contact SigmaAldrich.com/techservice.

This kit is for Research Use Only. Not for Use in Diagnostic Procedures.

Reagents

The SMC® Capture Labeling Kit includes all reagents listed in Reagents Provided. Additional reagents and supplies may be required to perform the labeling, as listed in the section titled General Supplies Required (Not Provided). All reagents supplied are for Research Use Only.

Reagents Provided

All products are available at SigmaAldrich.com unless otherwise noted.

Description	Storage Conditions	Packaging Details	Component Number
Capture Label	≤ –70 °C	1 x 20 µL	02-0557-00
Buffer 1	2-8 °C	1 x 25 mL	02-0552-00
Buffer 2	2-8 °C	1 x 500 µL	02-0553-00
Buffer 3	2-8 °C	1 x 5 mL	02-0554-00
Filter Tube Ultrafree®	2-8 °C	2 pack	02-0555-00
Amicon® Ultra-4 Filter Tube with Ultracel®-30 membrane	2-8 °C	2 pack	02-0556-00
10X Wash Buffer	2-8 °C	1 x 50 mL	02-0001-03
Coated Bead Buffer	2-8 °C	1 x 5 mL	02-0558-00
Uncoated SMC® Beads	2-8 °C	1 x 4 mL	02-0559-00

Note: Additional Amicon® Ultra-4 Centrifugal Filter Units with Ultracel®-30 membrane can be purchased from SigmaAldrich.com (Cat. No. UFC803008).

Storage Instructions

- The SMC® Capture Labeling Kit should be stored at 2-8 °C.
- The Capture Label should be stored at ≤ –70 °C.
- Proper kit performance can only be guaranteed if the materials are stored properly.
- 10X Wash Buffer does not contain preservative. After dilution, the 1X wash buffer may be filter sterilized with Stericup® Filter for storage of up to 1 month at 2-8 °C. If not filter sterilized, all remaining 1X Wash Buffer should be discarded upon experiment completion.

Additional Supplies Required (Not Provided)

- De-ionized or distilled water (DI Water)
- Pipettes capable of transferring 5 µL-5 mL
- Polypropylene Eppendorf® tubes
- Centrifuge with swinging buckets (capable of holding 15 mL conical) that can spin at 3900 x *g* (3900 RCF)
- Spectrophotometer or nanodrop (capable of reading A280)
- Bench top vortex
- Bench top mini-centrifuge
- 15 mL and 50 mL conical tubes
- Sodium azide (NaN_3) (optional)

Please contact your technical services representative for additional information or assistance selecting required but not provided supplies.

Precautions

Use caution when handling biological samples. Wear protective clothing and gloves.

Components of this reagent kit contain sodium azide as a preservative. Sodium azide is a toxic and dangerous compound when combined with acids or metals. Solutions containing sodium azide should be disposed of properly.

Full Hazardous Label

Ingredient	Cat. No.	Label	
10X Wash Buffer	02-0001-03		**Warning.** Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Capture Label	02-0557-00		**Warning**. Combustible liquid.

Capture Antibody Labeling

Preparation of Capture Antibody for Labeling

Antibodies need to be unlabeled and free of protein carrier such as BSA as well as supplemental reagents such as gelatin and glycerol to ensure success. Obtain carrier-free antibodies or purify these materials before proceeding.

Some antibody stabilizers (including sodium azide) and amine-containing buffers (including Tris) will significantly reduce capture labeling. Follow the steps below to remove these prior to antibody coupling.

1. Bring all reagents to room temperature.
2. Dilute antibody to approximately 1 mg/mL in Buffer 1.
3. Confirm the antibody concentration by reading the absorbance at 280 nm as follows:
   - Prepare 100 µL of a 1:10 dilution of the 1 mg/mL antibody solution in Buffer 1.

     Note: This is based on a spectrophotometer. Ab can be measured neat if using a nanodrop.
   - Measure the absorbance (using Buffer 1 as a blank).
   - Using Beer's Law A = εLc, calculate the antibody concentration as shown below. The example assumes a 1 cm path length and a molar extinction coefficient of 1.4:

     $$\text{Conc. (mg/mL)} = \frac{A280}{1.4} \times 10$$

     (For example, if A280 = 0.12 the concentration is (0.12/1.4) * 10 = 0.86 mg/mL)
4. Use the calculated antibody concentration to calculate the volume of antibody solution needed to label 0.2-1 mg Antibody. (For example, to label 200 µg of Ab: 0.20 mg/0.86 mg/mL = 232 µL Ab.
5. Prepare the Ultra-4 30K filter tube:
   - Pre-wet the Ultra-4 30K filter tube by adding 4 mL Buffer 1.
   - Centrifuge for 5 minutes at 3900 x *g* (3900 RCF). Discard the flow through.
6. Label two 15 mL conical tubes W1 and W2. Add the antibody volume calculated in step 4 to the Ultra-4 30K filter tube, W1. Bring the volume up to 4 mL with Buffer 1. Centrifuge for 10 minutes at 3900 x *g* (3900 RCF). Save the flow through in conical tube W1.

   (For example, 0.232 mL Ab + 3.76 mL Buffer 1 = 4 mL total volume.)

8. Transfer the filter containing the antibody into conical tube W2 and bring the volume up to 4 mL with Buffer 1. Centrifuge for 10 minutes at 3900 x *g* (3900 RCF). Save the flow through in conical tube W2.

9. Mix and remove the concentrated antibody from the filter tube and transfer to an Eppendorf® Tube. Determine the approximate volume remaining.

   Tip: Use the Buffer 1 to rinse the membrane to recover more antibody.

10. Calculate the volume of Buffer 1 required bringing the antibody volume back to approximate volume at the start of the buffer exchange.

11. Verify the antibody concentration by reading the absorbance at A280 (using Buffer 1 as the blank).

    Note: The concentration should be similar to the initial A280 reading. After the concentration of antibody is confirmed to be close to the initial amount, discard the W1 and W2 flow through tubes. If antibody yield is low, use W1 and W2 to extract the antibody by using new filters.

Label Capture Antibody

Calculate the volume of Capture Label required as follows:

$$\text{Capture Label (µL)} = 6 \times \text{Ab (mg)}$$

Note: The volume of capture label is 6 µL per 1 mg antibody.

For example, to label 200 µg of antibody requires (6 x 0.20) = 1.2 µL capture label.

1. Add the calculated volume of Capture Label to the antibody tube. Vortex and incubate for 1 hour at room temperature.
2. During the incubation, prepare 1X Buffer 3 by diluting the 5 mL of the 10X Buffer 3 into 45 mL of DI water.
3. After incubation, calculate the volume of Buffer 2 required to the labeled antibody to quench the reaction.

   Note: The volume of Buffer 2 is 5x the volume of Capture Label. Mix by vortex. (For example, Buffer 2: 1.2 µL x 5 µL= 6 µL)

   Tip: Excess can be added to round up (For example, 10 µL instead of 6 µL).

$$\text{Buffer 2 (µL)} = \text{Capture Label (µL)} \times 5$$

Prepare the Ultra-4 30K filter tube

1. Pre-wet the Ultra-4 30K filter tube by adding 4 mL Buffer 3.
2. Centrifuge for 5 minutes at 3900 x *g* (3900 RCF). Discard the flow through.
3. Label four 15 mL conical tubes W1, W2, W3 and W4.
4. Add the antibody volume to the Ultra-4 30K filter tube, W1. Bring the volume up to 4 mL with Buffer 3. Centrifuge for 10 minutes at 3900 x *g* (3900 RCF). Save the flow through in conical tube W1.
5. Repeat the buffer exchange with 1X Buffer 3 three more times and save the flow through in tubes W2, W3, and W4.
6. Remove the labeled antibody from the filter (W4) and dilute to approximately 1 mg/mL in Buffer 3.
7. Verify the antibody concentration by reading the absorbance at A280 (use 1X Buffer 3 as the blank).

   Note: The concentration should be similar to the initial A280 reading. If the concentration is acceptable, discard W1-W4 flow through.

   Tip: To retain most of the Ab, you can use the undiluted Ab to take the measurement.
8. Filter the labeled antibody using the Ultrafree® filter and a minicentrifuge for two minutes at maximum speed. Label and store the antibody at 4 °C.
9. If desired, add NaN_3 to 0.1% as a preservative.

Coating of Microparticle Beads with Capture Antibody

This procedure is for the intended purpose of coating magnetic microparticle (MP) beads with the labeled capture antibody.

Preparation and Coating Microparticle Beads for Conjugation

1. Rotate the uncoated MP bead vial at room temperature for 30 minutes to fully resuspend the beads.
2. During the rotation, prepare 1X Wash Buffer by diluting the 5 mL of the 10X Wash Buffer into 45 mL of DI water.
3. Calculate the volume of labeled capture antibody required to coat at 12.5 µg IgG/mg beads and 25 µg IgG/mg bead.

   Note: the concentration of uncoated MP beads provided is 10 mg/mL, therefore, 100 µL is 1 mg of MP beads.

   $$\text{Vol}(\mu L): \quad \frac{\text{Capture coating } (\mu g)}{\text{Ab. Conc. } (mg/mL)} \times 1 \text{ mg of MP bead}$$

   (For example: To label 2 mg of MP bead at 12.5 ug coating, (12.5/0.86) x 2 = 29 µL capture Ab. For 25 ug coating, 29 x 2 = 58 µL of capture label.)

Wash the MP beads as follows:

4. Label two Eppendorf® Tubes, 12.5 µg and 25 µg.
5. Place the tube(s) containing the beads in a bench top magnetic bead separator.
6. Allow beads to sit in magnet for ≥ 2 minutes. Visually confirm that all beads have been pulled toward the magnet.
7. Aspirate the supernatant with a pipette and discard.
8. Add 1 mL of 1X Wash Buffer to the tube(s). Turn the tube in the magnet holder 180° several times to wash the beads.
9. Allow beads to sit in magnet for ≥ 2 minutes, then remove and discard the Wash Buffer.
10. Repeat the wash step two more times using fresh 1X Wash Buffer.
11. Aspirate and discard the 1X Wash Buffer.

12. Add the calculated volume of capture antibody to each of the tubes, followed by the calculated volume of wash buffer based on the mg of beads being labeled.

    Total Vol (µL): = Capture (µL) + Wash Buffer (µL)

    (For example: To label 2 mg of MP bead at 12.5 ug, 200 µL MP bead – 29 µL capture antibody = 171 µL Wash Buffer.)

13. Incubate for 60 minutes at room temperature with gentle inversion on a rotator.

14. After incubation, wash the beads by placing in a bead magnetic separator and repeat wash step 4, five times.

After final wash resuspend antibody coated MP beads in Coated Bead Buffer as follows:

15. After final wash in above step allow beads to sit in magnet for ≥ 2 minutes.

16. Visually confirm that all MPs have been pulled to the magnet.

17. Aspirate the supernatant with a pipette and discard.

18. Add equal amount of Coated Bead Buffer to resuspend and block the coated beads at a 10 mg/mL. For example, 2 mg (200 µL) of beads requires 200 µL of Coated Bead Buffer.

19. Either block the coated beads for 1hr at RT or overnight at 4 °C rotating.

20. Store the blocked beads at 4 °C until use.

Worksheet

See preceding protocol for more detailed information on each step.

Pre-Label

Step	Result
Dilute Antibody to ~1 mg/mL in Buffer 1	Vol. added: _____ mL
Read A280 of antibody	A280: _____ Conc. (C): _____ mg/mL
Determine amount and volume of antibody to label	Ab Amount (A): _____mg (up to 1 mg) Volume (A/C): _____ mL
Rinse Ultra-4 30K filter tube with 4 mL Buffer 1, 5 minutes at 3900 RCF	
Add Antibody to Ultra-4 30K filter tube	
Wash #1 with Buffer 1 (10 min.)	Ab. Vol.: _____ mL + _____mL Buffer = 4 mL
Wash #2 with Buffer 1 (10 min.)	Up to 4 mL Buffer 1
Dilute to ~1.0 mg/mL in Buffer 1	Recovered Vol: _____ mL Buffer 1 added: _____ mL
Read A280 of antibody	A280: _____ Conc. (C): _____ mg/mL

Labeling

Step	Result
Add Capture Label. Mix by vortexing.	Capture Label added = _____ µL
Incubate 1 hour at RT.	Start time: _____ End Time: _____

Post-label

Step	Result
Rinse new Ultra-4 30K filter tube with 4 mL 1x Buffer 3, 5 minutes at 3900 RCF	
Add labeled Ab to Ultra-4 30K filter tube	Ab vol: _____ mL
Wash #1 with 1X Buffer 3 (10 min.)	Ab vol: _____ mL + _____ mL Buffer 3 = 4 mL
Wash #2, #3, #4 with 1X Buffer 3 (10 min.)	Up to 4 mL Buffer 3
Dilute the antibody to approx. 1 mg/mL with 1X Buffer 3	Buffer 3 added: _____mL
Read A280 of antibody	A280: _____ Conc. (C): _____ mg/mL

MP Conjugation

Step	Result
Wash uncoated beads with 1X Wash Buffer 3 times.	
Add calculated volume of Antibody to coat at 12.5 and 25 µg/IgG/mg bead.	Ab vol. added to coat 12.5 µg: _____µL Ab vol. added to coat 25 µg: _____µL
Incubate 1 hr at RT, rotating.	Start time: _____ End Time: _____
Wash beads with 1X Wash Buffer 5 times	
Add calculated amount of coated bead buffer to block beads at RT for 1 hr, or overnight at 4 °C rotating.	Start time: _____ End Time: _____

Product Ordering

Products are available for online ordering at SigmaAldrich.com.

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