Erenna®

SMC™ Detection Reagent Labeling Kit Instructions

Detection Labeling Kit

Catalog # 03-0076-02

Kit for the fluor labeling of the Detection Reagent for use with SMC™ **Analyte Specific Immunoassays**

FOR RESEARCH USE ONLY

NOT FOR USE IN DIAGNOSTIC PROCEDURES

Manufactured & Distributed by:

EMD Millipore Corporation
3050 Spruce Street
St. Louis, Missouri 63103
United States of America
emdmillipore.com

This page intentionally left blank.

TABLE OF CONTENTS

INTRODUCTION

The Erenna® SMC™ Immunoassay uses a quantitative fluorescent sandwich immunoassay technique to measure analyte in a matrix. This kit is intended for use with the SMC™ Erenna and is for the specific purpose of labeling up to 1mg of an analyte specific detection Antibody (Ab). The eluted sample is loaded onto the Erenna® Immunoassay System where the labeled molecules are detected and counted. The number of fluor-labeled detection Ab counted is directly proportional to the amount of analyte present in the sample when captured. This kit should be used in conjuction with 03-0077-02 Erenna® SMC™ Capture Reagent Labeling Kit and 03-0078-00 Erenna® SMC™ Bead Based Immunoassay Development Kit**.**

Antibodies used are to be in carrier- and label-free buffer.
Minimum Detection Antibodies Required 0.2 mg up to 1 mg.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

REAGENTS

The SMC™ Detection Reagent Labeling Kit includes all reagents listed in Reagents Provided. Additional reagents and supplies may be required to run this immunoassay, as listed in the section titled General Supplies Required But Not Provided. All reagents supplied are for Research Use Only.

Reagents Provided

Item #	Description	Shipping Conditions	Storage Conditions	Component Part No.	Packaging Details
1	Detection label	Dry ice	≤ -70ºC	02-0574-00	1 x 20 µL
2	Buffer 1	With cold pack	2-8ºC	02-0552-00	1x 25 mL
3	Buffer 2	With cold pack	2-8ºC	02-0553-00	1 x 500 µL
4	Buffer 3	With cold pack	2-8ºC	02-0554-00	1 x 5 mL
5	Filter Tube Ultrafree	With cold pack	2-8ºC	02-0555-00	2 Pack
6	Ultra 4 Filter Tube	With cold pack	2-8ºC	02-0556-00	2 Pack

Storage Instructions

The SMC™ Detection Reagent Labeling Kit should be stored at 2 - 8°C.
The Fluor should be stored at or below ≤ -70°C.
Proper kit performance can only be guaranteed if the materials are stored properly.

General Supplies Required But Not Provided

- De-ionized or distilled water (DI Water)
- Pipettes capable of transferring 5 µL- 5 mL
- Micro-centrifuge tubes
- Centrifuge with swinging buckets (capable of holding 15 mL conical) that can spin at 3900 x g (3900 RCF)
- Spectrophotometer (capable of reading A280 and A650)
- Bench top vortex
- Bench top mini-centrifuge
- Vial, 0.5 mL and 2 mL for storage of labeled Antibody
- Tube, PP, 15 mL, 50 mL Conical
- Cap, Screw, 0.5mL
- Sodium Azide (NaN_3) (optional)

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

TECHNICAL HINTS DUE TO HIGH SENSITIVITY

To obtain reliable and reproducible results, the operator should carefully read this entire manual and fully understand all aspects of each assay step before running the assay. The following notes should be reviewed and understood before the assay is set-up.

- Wipe down bench and pipettes with 70% isopropanol before use. It is important to allow all reagents to warm to room temperature (20 - 25°C).
- Quickly spin concentrated Fluor before opening vials.
- Pre-wet tips (aspirate and dispense within well) twice before each transfer.

PRECAUTIONS

- Use caution when handling biological samples. Wear protective clothing and gloves.
- Components of this reagent kit contain approximately 0.08% 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 #		Full Label	
02-0574-00	Detection Label		**Warning.** Combustible liquid.

LABELING THE DETECTION ANTIBODY (Using 1mg/mL Ab)

1) Prepare a 1X solution of Buffer 3 in a 50 mL Conical tube by adding 5 mL of 10X Buffer 3 to 45 mL DI water.
2) It is recommended that antibodies are carrier free for the labeling precedure. Dilute or prepare a 1 mg/mL concentration solution of Ab in 1X Buffer 3.
3) Confirm the Ab concentration by reading the absorbance at A_{280} as follows:

    a. Prepare 100 µL of 1:10 dilution of the 1mg/mL Ab solution in 1X Buffer 1 and measure the absorbance using a 1 cm path cuvette (make sure to use Buffer 1 as blank).

    Using Beer's Law formula, A=εLC calculate the Ab concentration as shown below:

> **Ab Conc. (mg/mL) = (A_{280} x 1 mg/mL/1.4) x 10**
>
> *e.g.* If the A_{280} = 0.099, then Ab conc, (mg/mL) is as follows,
>
> Ab Conc. (mg/mL) = (0.099 x 1 mg/mL/1.4) x 10 = 0.71 mg/mL
>
> *Note: For most typical 1 mg/mL antibody solutions, A_{280} is assumed to be 1.4*

    b. Using the calculated Ab concentration above, calculate the volume of Ab solution required by using the formula below to obtain a 1 mg solution:

> Actual Ab Conc. (mg/mL) x Ab (mL)= 1 mg
>
> Ab mL = 1 mg / Actual Ab Conc. (mg/mL)
>
> *e.g.* If actual concentration of Ab is 0.71 mg/mL, then the volume needed is:
>
> Ab (mL) = 1 mg / 0.71 mg/mL = 1.41 mL of Ab

4) Buffer exchange using Buffer 1 (twice) and 1 mg of antibody as follows:
    a. Pre-wet the Ultra 4 30K filter tube by adding 4 mL Buffer 1.
    b. Centrifuge for 5 minutes at 3900 x g (3900 R.C.F.). Discard the flow through.
5) Add 1 mg of the antibody (volume calculated in step 3b) to the Ultra 4 30K filter tube.
6) Calculate the volume of Buffer 1 required to bring the total volume to 4 mL.
    - 4 mL - Ab (mL)= buffer (mL)

      E.g. 4 mL - 1.41 mL= 2.59 mL

LABELING THE DETECTION ANTIBODY (continued)

7) Label the Ultra - 4 30K filter tube W1 and centrifuge for 10 minutes at 3900 x g (3900 R.C.F).Save the flow through in the conical tube marked W1.
8) Place the Ultra - 4 30K filter tube in a new 15mL conical tube labeled W2. Add Buffer 1 to bring the volume to 4mL. Centrifuge for 10 minutes at 3900 x g (3900 R.C.F). Save the flow through in W2 conical tube.
9) Mix and remove the concentrated antibody from the filter tube and add into an Eppendorf tube.
10) Add Buffer 1 to bring the concentration of the Ab back to approximately 1 mg/mL.

    E.g. If the volume of Ab is 0.25 mL (after final buffer exchange), then add 0.75 mL Buffer 1. Remember the starting amount of Ab was 1mg at the beginning of the buffer exchange

11) Follow the steps below to verify the actual Ab concentration by reading the absorbance at A_{280} (make sure to use Buffer 1 as blank).

> *Note: Use calculation from step 3 to calculate the Ab concentration. Also note that there is no 1:10 dilution.*
>
> *e.g.* If the measured A_{280} =1.315, then Ab conc, (mg/mL) is as follows,
>
> Ab Conc. (mg/mL) = 1.315 x 1 mg/mL/1.4 = 0.94 mg/mL

Optional: you may save the volume of Ab solution used for reading A_{280}.

After the concentration of antibody is confirmed to be close to 1 mg/mL, discard W1, W2 flow through. If antibody yield is low, use W1 and W2 to extract the antibody by using new filters.

12) Calculate the volume of Detection Label required for conjugation as follows:

> (detection label in µL) = 15 µL/(mg of Ab) x Ab (mg)
>
> *e.g.* detection label (µL) = 15 (µL/mg Ab) x 0.94 (mg Ab) = 14.1 µL
>
> *Note: The required volume of detection label is 15 µL per 1mg of Ab.*

13) Add the calculate label volume to Eppendorf tube with Capture Ab and immediately mix by vortexing.

LABELING THE DETECTION ANTIBODY (continued)

14) Incubate 1 – 2 hours at room temperature

Start time: _____ End time: _____

15) At the end of incubation, calculate the volume of Buffer 2/Buffer C to be added to the labeled Ab tube:

Buffer 2/Buffer C (µL) = 3.75 x detection label (µL)

e.g. Buffer 2/Buffer C (µL) = 3.75 x 14.1 µL= 52.875 µL

Note: The required volume of Buffer 2/Buffer C is 3.75 times the volume of detection Label.

16) Add Buffer 2 to the tube containing the labeled Ab and mix by vortexing.

17) Pre-Wet a new Ultra 4 30K filter tube with Buffer 3, as follows:

a. Place the Ultra 4 30K filter tube in a conical tube and add 4 mL 1X Buffer 3.

b. Centrifuge for 5 minutes at 3900 x g (3900 R.C.F). Discard the flow through.

18) Perform Buffer exchange with 1X Buffer 3 (four times) as follows:

a. Transfer the labeled antibody into the prepared Ultra 4 30 K filter tube.

b. Place the Ultra 4 - 30K filter tube into a new conical tube marked W1. Add 1X Buffer 3 to bring the volume to 4 mL. Centrifuge for 10 minutes at 3900 x g (3900 R.C.F). Save the flow through in the conical tube marked W1.

c. Repeat the buffer exchange with 1X Buffer 3 three more times and save the flow through in tubes marked W2, W3 and W4.

19) Read A650 of Wash 4. If < 0.1 proceed to Ultrafree filtration. If > 0.1, continue to wash 5.

20) Measure the A_{280} of W1-W4 and if no Ab loss observed discard and proceed.

21) If any of the W1-W4 shows Ab, repeat buffer exchange using new Ultra 4 30K filter tube.

LABELING THE DETECTION ANTIBODY (continued)

22) Dilute the labeled Ab to 1mg/mL in 1X Buffer 3. Assuming that you did not lose any antibody, using the Ab concentration (mg/mL) obtained in step 15, add enough 1X Buffer 3 to prepare the 1mg/mL labeled Ab. *Note: if desired add 0.1 % NaN_3 as preservative.*

> *E.g.* Ab Conc. (mg/mL) = 0.94 mg/mL from step 15
>
> Therefore, add enough 1X Buffer 3 to the filter tube to bring the volume to 0.94 mL. This volume is assumed no NaN_3 will be added

23) Filter the labeled Ab by using the Ultrafree filter and a minicentrifuge. Label and store the Labeled Ab at 4-8°C.

ORDERING INFORMATION

To place an order or to obtain additional information about SMC™ products, please contact your Customer Service or Technical Support Specialist.

Contact information for each region can be found on our website:

emdmillipore.com/contact

Conditions of Sale

For Research Use Only. Not for Use in Diagnostic Procedures.

Safety Data Sheets (SDS)

Safety Data Sheets for EMD Millipore products may be ordered by fax or phone or through our website at emdmillipore.com/msds

EMD Millipore Corporation
3050 Spruce Street
St. Louis, Missouri 63103
United States of America

Toll-Free US: (800) 645-5476
Fax: (800) 645-5439
emdmillipore.com