CellMosaic™

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Streptavidin-Antibody Conjugation Kit (CM52420x1 and CM52420x3) User Reference Guide

Contents

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Important Notes & Contact Information

READ BEFORE USING ANY RESOURCES PROVIDED HEREIN

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Sample data are provided for illustration and example purposes only and represent a small dataset used to verify kit performance in the CellMosaic laboratory. Information about the chemicals and reagents used in the kit are provided as necessary.

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Kit Components

This kit provides materials to perform streptavidin labeling of one (CM52420x1) or three (CM52420x3) antibody samples. Scale of each reaction: 1 mg antibody (protein content). Upon receipt, please remove the plastic bag containing activated streptavidin and Reagents A, B, and C and store in a freezer less than -20°C. Store the rest of the items and box in a refrigerator at 2-8°C.

Name	Part #	Quantity (CM52420x1)	Quantity (CM52420x3)	Storage condition
Activated Streptavidin (red label)	CM52104	2 x 1 mg	6 x 1 mg	-20°C
Reagent A (cyan label)	CM12101	1 unit	3 units	-20°C
Reagent B (blue label)	CM12005	1 unit	3 units	-20°C
Reagent C (yellow label)	CM13001.1	1 unit	3 units	-20°C
Buffer A (orange label)	CM02001	8 mL	24 mL	2-8°C
Buffer B (indigo label)	CM02005	12 mL	36 mL	2-8°C
PBS Buffer (grey label)	CM02013	5 mL	20 mL	2-8°C
Centrifugal Filter Device 1	CM03CD050A	2	6	2-8°C
Centrifugal Filter Device 2	CM03CD010A	1	3	2-8°C
Centrifugal Filter Device 3	CM03CD100A	1	3	2-8°C
Desalting Column	CM03SG05	1	3	2-8°C
Spin Column (pyridyl dithiol agarose)	CM71606	2	6	2-8°C
1.5 mL Centrifuge Tube	N/A	4	12	2-8°C
Collection Tubes	N/A	4	12	2-8°C
Antibody IgG	N/A	NOT PROVIDED (User Supplied Material, 1 mg for each reaction)		

Safety Information

Warning: Some of the chemicals used can be potentially hazardous and can cause injury or illness. Please read and understand the Material Safety Data Sheets (MSDS) available at CellMosaic.com before you store, handle, or use any of the materials.

Labeling Chemistry

The kit is designed to work with antibody IgG. The user supplies their own unmodified IgG. Using the kit components, the user converts the antibody to a thiol-antibody, followed by reaction of the thiol-antibody with activated streptavidin to generate the streptavidin-antibody conjugates. The combination of filtration and scavenger type purification steps typically provides the resulting streptavidin-antibody at greater than 90% purity.

Key features of this streptavidin-antibody conjugation kit:

- High quality maleimide activated streptavidin for the conjugation: >99% purity
- Average 3 streptavidin molecules per antibody for a typical batch

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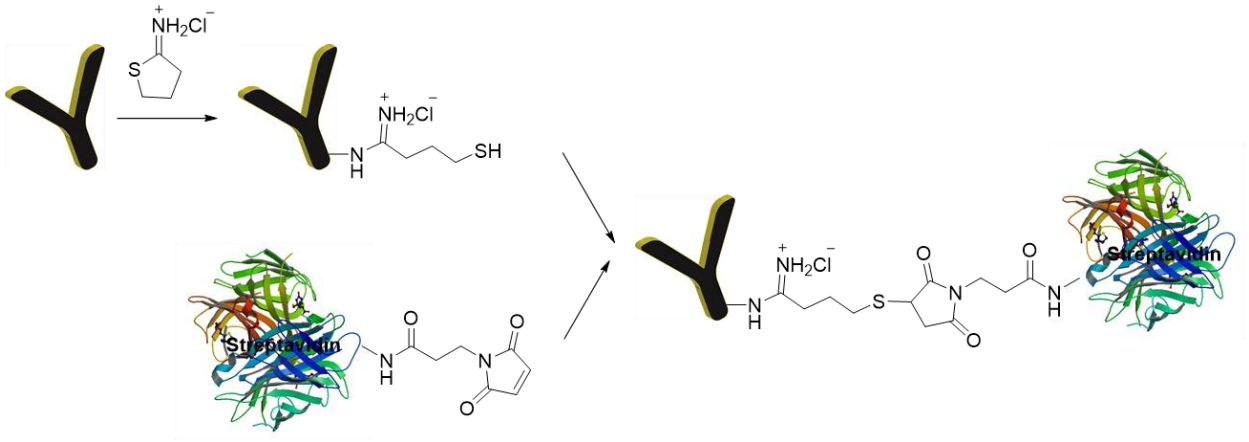
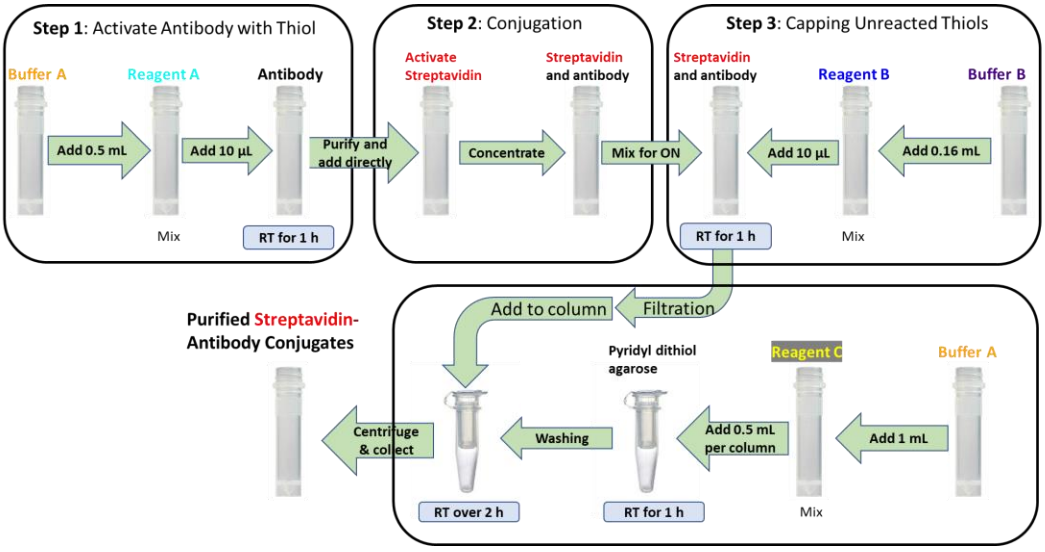
- Over 90% pure streptavidin-antibody conjugates after purification
- All reagents included, from preparation to purification



Requirement for antibody (IgG):
1. Preferably, the antibody should be >90% pure by gel electrophoresis
2. Total amount: 1 mg (protein content)

Potential interfering compounds for labeling and conjugation reactions: *Thiols*, e.g., DTT and mercaptoethanol

Protocol



Scheme 1. Schematic diagram of the work flow for preparing streptavidin-antibody conjugates

1. Lab Instrumentation Needed

- Vortex mixer, centrifuge (preferably refrigerated)

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- Pipettes and tips
- Timer
- Incubator or shaker set at 37°C or RT.
- Chemical hood
- Support stand, lab frame, or any support rod for desalting column
- Flask
- Personal protection equipment (lab coat, safety glasses, and chemical resistant nitrile gloves)
- UV spectrophotometer (optional)

2. Preparation of Antibody Samples for Conjugation

Total amount of antibody used for the conjugation is 1 mg (protein content measured by UV).

A1. Insert **Filter Device 1** (CM03CD050A) into one of the provided collection tubes (microcentrifuge tube with the cap attached). Perform the step based on the following conditions.

- ✓ If your antibody is supplied as a lyophilized solid, dissolve the antibody in 500 µL of **deionized water** and then transfer the entire contents to **Filter Device 1**.
- ✓ If your antibody is supplied in < 500 µL buffer, transfer your antibody sample to the **Filter Device** directly. Add **Buffer A** (orange label) to make up the total volume to 500 µL and cap it.
- ✓ If the volume of your antibody sample is between 500 and 1000 µL, divide the volume into two of the **Centrifugal Filter Device 1** and add the antibody sample to the filter device. Add **Buffer A** to make up the total volume to 500 µL in each device and cap them.
- ✓ If the volume of your antibody sample is >1000 µL, add up to 500 µL of sample to the two **Filter Devices** and cap them. Repeat Step **A1-A4** until all of the antibody sample goes into the **Filter Device**. Move on to Step **A5**. Add **Buffer A** to make up the total volume to 500 µL in each device for the last refill.

A2. Place the capped **Filter Device** into the centrifuge rotor, aligning the cap strap toward the center of the rotor; counterbalance with a similar device.

A3. Spin the **Filter Device** at 14,000 x g for 8 minutes (preferably cooled to 4°C) to concentrate to **< 100 µL.** (Spin time depends on many factors. The typical spin time for a 500 µL sample is approximately 8 to 20 minutes. The typical volume is ~40 µL after spinning for 8 minutes on an Eppendorf 5417R at 4°C.)

A4. Remove the assembled device from the centrifuge and separate the **Filter Device** from the collection tube. Transfer the filtrate from the collection tube to a clean centrifuge tube (not provided). **Save the filtrate until the experiments are done.**

A5. Insert the **Filter Device** back into the collection tube. Add 400-450 µL of **Buffer A** to make up the total volume to 500 µL. Then place the capped **Filter Device** into the centrifuge rotor,

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aligning the cap strap toward the center of the rotor; counterbalance with a similar device. Spin the device at 14,000 x g to concentrate to **< 100 µL**. Remove the assembled device from the centrifuge and separate the **Filter Device** from the collection tube. Transfer the filtrate from the collection tube to a clean centrifuge tube (not provided). **Save the filtrate until the experiments are done.**

A6. Repeat **Step A5** two more times.

> **Note**: If you divide your samples into two **Centrifugal Filter Devices,** you can combine the samples into one **Centrifugal Filter Device** during the last repeat of **Step A6**.

A7. Transfer the concentrated sample from the **Filter Device** to a 1.5 mL micro-centrifuge tube (use a pipetman to measure the approximate volume of the concentrated sample).

A8. Add 50 µL of **Buffer A** to the **Filter Device** to rinse. Stir it gently with a pipet tip, then transfer the entire contents to the 1.5 mL micro-centrifuge tube from **Step A7.**

A9. Repeat **Step A8** once. Add **Buffer A** to make up the total volume of the sample to ~200 µL and cap it.

A10. Vortex the combined antibody sample for 30 seconds and then centrifuge to ensure no liquid is in the cap.

3. Activate Antibody with Thiol

B1. Briefly spin the tube containing **Reagent A** (cyan label). Add 0.5 mL of **Buffer A** to the tube with **Reagent A**. Vortex for 30 seconds to 1 minute to dissolve the reagent.

> **Tip for solubility check**: Check the bottom of the micro-centrifuge tube to see if the solution is clear and free of any solid residue.

B2. Transfer 10 µL **Reagent A** solution from **Step B1** to the 1.5 mL micro-centrifuge tube containing antibody solution from **Step A10**.

B3. Vortex the solution for 30 seconds, and then centrifuge to ensure no liquid is in the cap. Mix the reaction mixture at RT for 1 hour.

> **Tip for mixing**: You can use a nutator, a shaker, a vortex, or an incubator shaker for mixing. If you are using end to end nutating, make sure your centrifuge is capped properly. If you don't have any of this equipment, you can let the centrifuge tube sit at the bench with manual mixing by pipetting every 20 minutes.

4. Purification to Remove Excess Reagent A and Conjugation with Streptavidin

C1. Securely attach the **Desalting Column** to a support stand, a lab frame, or any support rod. Remove the top and bottom caps from the column and allow the excess liquid to flow through by gravity. Collect the liquid in a flask.

C2. Add 2.5 mL of **Buffer B** (indigo label) and allow the buffer to completely enter the gel bed by gravity flow.

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C3. Repeat **Step C2** two times.
C4. Spin the thiol-modified antibody from **Step B3** to ensure there is no liquid in the cap before opening it. Add the entire antibody solution to the column. Allow the sample to enter the gel bed completely.
C5. Add 290 µL of **Buffer B** and allow the buffer to completely enter the gel bed by gravity flow.
C6. Place one of the tubes containing 1 mg of **Activated Streptavidin** (red label, total two tubes) under the column. Add 355 µL of **Buffer B** to the column. Collect the eluent by gravity and allow the buffer to enter the gel bed completely.
C7. Place the other tube containing 1 mg of **Activated Streptavidin** under the column. Add 355 µL of **Buffer B** to the column. Collect the eluent by gravity and allow the buffer to enter the gel bed completely.
C8. Vortex the eluent from **Steps C6** and **C7** for 30 seconds to 1 minute to dissolve the streptavidin. Spin the reaction mixture to ensure there is no liquid in the cap before opening it.
C9. Insert **Filter Device 2** (CM03CD010A) into one of the provided collection tubes. Transfer up to 500 µL of the reaction mixture from Step **C8** to the **Filter Device**. Then place the capped **Filter Device** into the centrifuge rotor, aligning the cap strap toward the center of the rotor; counterbalance with a similar device. Spin the device at 14,000 x g for 5 minutes (preferably cooled to 4°C) to concentrate to < 150 µL. (The typical volume is ~120 µL after spinning for 6 minutes on an Eppendorf 5417R at 4°C).

C10. Transfer the rest of the reaction mixture from Step **C8** to the **Filter Device**. Washing the two tubes from **Step C8** with 100 to 150 µL of **Buffer B**. Then transfer the washing buffer to the Filter Device. Spin the device at 14,000 x g for 5 minutes (preferably cooled to 4°C) to concentrate to < 150 µL.

C11. Transfer the concentrated sample from the **Filter Device** to a 1.5 mL micro-centrifuge tube.
C12. Add 50 µL of **Buffer B** to the **Filter Device** to rinse. Stir it gently with a pipet tip, then transfer the entire contents to the **micro-centrifuge tube** from **Step C11**.
C13. Repeat **Step C12** once.
C14. Add **Buffer B** to make up the total volume of the samples to 250 µL. Vortex the solution for 30 seconds, and then centrifuge to ensure no liquid is in the cap. Mix the reaction mixture at RT for overnight (16 to 20 hours).

5. Capping Unreacted Thiol Groups of Antibody

D1. Briefly spin the tube containing **Reagent B solution** (blue label). Add 160 µL of **Buffer B** to the tube containing **Reagent B**. Vortex for 30 seconds to 1 minute to mix, and then centrifuge to ensure no liquid is in the cap.
D2. Transfer 10 µL **Reagent B** solution from **Step D1** to the micro-centrifuge tube containing the reaction mixture from **Step C14**.
D3. Vortex the solution for 30 seconds, and then centrifuge to ensure no liquid is in the cap. Mix the reaction mixture at RT for 30 minutes to 1 hour.

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6. Purification to Remove Excess Reagent B and Streptavidin

Note: Steps **E1** to **E5** are for activating the thiols at agarose beads.

E1. Remove the pyridyl dithiol agarose spin columns from the plastic bag. Place the two capped spin columns into the centrifuge rotor, aligning the cap strap toward the center of the rotor. Spin the columns at 3,000 x g for 1 minute. Separate the column from the collection tube. Discard the filtrate.

E2. Insert the column back to the collection tube. Add 500 µL of **Buffer A**. Spin the columns at 3,000 x g for 1 minute. Separate the column from the collection tube. Discard the filtrate.

E3. Repeat Step E2 two times (total 3 washings).

E4. Briefly spin the tube containing **Reagent C** (yellow label). Add 1 mL of **Buffer A** to the tube containing **Reagent C**. Vortex for 30 seconds to 1 minute to dissolve the solid, and then centrifuge to ensure no liquid is in the cap.

E5. Transfer 500 µL of **Reagent C solution** from **Step E4** to each spin column. Cap the column and vortex the solution for 30 seconds, and then let it stand at RT for 1 hour.

Note: Steps **E6** to **E9** are for removing unreacted **Reagent B**.

E6. Insert **Filter Device 3** (CM03CD100A) into one of the provided collection tubes. Transfer the entire solution from Step **D3** to the **Filter Device.** Add 250 µL of **PBS buffer** (grey label) to make up the total volume to 500 µL and cap it. Then place the capped **Filter Device** into the centrifuge rotor, aligning the cap strap toward the center of the rotor; counterbalance with a similar device. Spin the device at 14,000 x g for **8 minutes**.

E7. Remove the assembled device from the centrifuge and separate the **Filter Device** from the collection tube. Transfer the filtrate from the collection tube to a clean centrifuge tube (not provided). **Save the filtrate until the experiments are done.**

E8. Insert the **Filter Device** back to the collection tube. Add 400-450 µL of **PBS buffer** to make up the total volume to 500 µL. Then place the capped **Filter Device** into the centrifuge rotor, spin the device at 14,000 x g for 8 minutes.

E9. Repeat **Step E7** two times (total 3 washings).

E10. Transfer the concentrated sample from the **Filter Device** to a 1.5 mL micro-centrifuge tube.

E11. Add 100 µL of **PBS Buffer** to the **Filter Device** to rinse. Stir it gently with a pipet tip, then transfer the entire contents to the 1.5 mL micro-centrifuge tube from **Step D6** and cap it.

E12. Repeat **Step E11** once.

E13. Vortex the combined conjugates for 30 seconds and then centrifuge to ensure no liquid is in the cap.

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Note: Steps **E13** to **E19** are for scavenging the unreacted streptavidin.

E14 (continue from Step E5). After 1 h, place the two capped spin columns from **Step E5** into the centrifuge rotor. Spin the columns at 3,000 x g for 1 minute. Separate the column from the collection tube. Discard the filtrate.

E15. Insert the column back to the collection tube. Add 500 µL of **Buffer B**. Spin the columns at 3,000 x g for 1 minute. Separate the column from the collection tube. Discard the filtrate.

E16. Repeat Step **E15** two times (total 3 washings).

E17. Wash the collection tube once with 500 µL of **PBS buffer**. Insert the column back into the empty collection tube. Divide the conjugates from **Step E13** into the two spin columns. Add 300-350 µL of **PBS buffer** to make up the total volume to 500 µL in each column.

E18. Cap the columns and vortex the solution for 30 seconds, and then let it stand at RT for minimum 2 hours (overnight is OK).

E19. Place the two capped spin columns from **Step E18** into the centrifuge rotor. Spin the columns at 3,000 x g for 1 minute. Separate the column from the collection tube.

E20. Transfer the filtrate from the collection tube to a 1.5 mL micro-centrifuge tube. Label it as product.

Streptavidin-Antibody is Ready for Your Experiment

Tip: The approximate concentration of the conjugate is 1-2 mg/mL in PBS buffer. The number of streptavidin molecules per antibody is 2-4 on average.

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Other Considerations

1. Concentration Determination

To determine the concentration, dilute your streptavidin-antibody with 1x PBS buffer. Measure the UV absorbance of streptavidin-antibody at 280 nm (A_{280}) using a UV spectrometer and calculate the concentration using the following formula:

$$Concentration\ (\mu M\ of\ Streptavidin) = \frac{\mathrm{A280}\ \times 10\mathrm{\hat{}}6}{\mathrm{L}\ \times (210000 + \mathrm{n} \times 41326)}$$

L: UV cell path length (cm). If you are using a 1 cm UV cell, you can dilute streptavidin-antibody 5 to 10 times to get a good reading.

n: average number of streptavidin molecules per antibody

Extinction coefficient of streptavidin is 41,326 $M^{-1}cm^{-1}$

Extinction coefficient of IgG (MW of 150,000) is 210,000 $M^{-1}cm^{-1}$

2. Analyze the Conjugate and Determine the Degree of the Streptavidin Loading by HPLC

The purity of the conjugate and average number of streptavidin molecules loaded onto the antibody can be analyzed by size exclusion HPLC. If you are familiar with HPLC, you can analyze them yourself. Alternatively, you can send the samples to CellMosaic for analysis on your behalf.

HPLC conditions

Buffer A: PBS buffer
Method: Isocratic
Flow rate: Will be determined by your column (usually 1 mL/min).
Injection amount for the conjugate: Dilute the sample 5 times with PBS buffer, then inject 10 µL

3. Recommended Storage Conditions

Depending on the stability of your antibody, streptavidin-antibody conjugate solution is recommended to be stored at 2-8°C and should be used as soon as possible. Some streptavidin-antibody conjugates may be lyophilized for long-term storage.

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Appendix: Typical Kit Performance Data (LC analysis, CellMosaic)

Antibody information: A therapeutic antibody (Human IgG1 subtype)

Kit Lot number: 5519.S10.080818

Figure 1: Size-exclusion HPLC analysis of the antibody (Ab, red), activated streptavidin (Strep, blue), and purified streptavidin-antibody conjugates (green).