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PerKit™ Antibody-Oligo Conjugation Kit (CM53405) User Reference Guide

Contents

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Important Notes & Contact Information

READ BEFORE USING ANY RESOURCES PROVIDED HEREIN

The information provided in this document and the methods included in this package are for information purposes only. CellMosaic provides no warranty of performance or suitability for the purpose described herein. The performance of labeling using this kit may be affected by many different variables, including but not limited to: purity and complexity of the antibody, differences in preparation techniques, operator abilities, and environmental conditions.

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 is provided as necessary.

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Kit Configuration and Components

This kit provides materials to perform one or three antibody oligo conjugations. Scale of each reaction: 0.25−1 mg antibody (protein content).

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If you purchased this kit previously, the current one is an upgraded and improved kit with slightly different components and workflow as the second-generation kit. This kit has multiple configurations for various lengths of oligos and can be used to conjugate **4–15** nano-mole (nmol) of oligo. **Table 1** provides the catalog numbers for various kit configurations and **Table 2** lists the kit components.

Table 1: Configurations of the PerKit™ Antibody-Oligo conjugation kit (CM53405).

Configuration	No. of Reactions	Catalog No.
1. Labeling for short oligo (5−30 bases)	1	CM53405.1x1
	3	CM53405.1x3
2. Labeling for medium oligo (31−59 bases)	1	CM53405.2x1
	3	CM53405.2x3
3. Labeling for long oligo (≥60 bases)	1	CM53405.3x1
	3	CM53405.3x3

How to use this protocol: The protocol in this user manual is written for three different configurations: 5−30 bases oligo (CM53405.1), 31−59 bases oligo (CM53405.2), and ≥60 bases oligo (CM53405.3). Steps are common to all configurations. However, some of the kit components are specific to each configuration.

⚠ Please follow the specific instructions for individual configurations.

Requirement for antibody (IgG):

1. Preferably > 90% pure by gel electrophoresis.
2. Total amount: 0.25−1 mg protein content as measured by UV. **Note:** the accuracy of your protein amount is important for obtaining optimized loading. Please refer to the section Other Considerations in this manual to measure the protein amount.

Requirement for amine-oligo (≥5-mer):

1. Total amount: 4−15 nmol oligo content as measured by UV. **Note:** the accuracy of your oligo amount is important for obtaining optimized loading. Please refer to the section Other Considerations in this manual to measure the oligo amount.
2. HPLC purified with amine-oligo content ≥90% by C18 HPLC.
3. ≥5-mer (oligos longer than 60-mer may result in lower loading and high impurities).

Note: You can purchase HPLC purified C6 amine-modified oligo from standard oligo manufacturers up to 90-mer length for this kit preparation. It is highly recommended that customers analyze and quantify oligos prior to using them.

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Table 2: Components and storage temperatures for PerKit™ Antibody-Oligo conjugation kit. All kits share the same components except for the number of tubes provided for EMCS and the type of Filter Devices.

- Upon receipt, please remove **Box 1** and store in a freezer at or below -20°C.
- Store **Box 2** in a refrigerator at 2–8°C.

Box No. (Storage T)	Name	Cat#	Kit Cat#	Quantity (x1)	Quantity (x3)
Box 1 (≤-20°C)	EMCS (**red label**)	CM12107.1	CM53405.1 CM53405.2	1 unit	3 units
			CM53405.3	2 units	6 units
	Reagent A (**cyan label**)	CM12101	Any	1 unit	3 units
	Reagent B Solution (**yellow label**)	CM12004.1	Any	1 unit	3 units
Box 2 (2–8°C)	Solution A (**Purple label**)	CM02057	Any	0.5 mL	0.5 mL
	Buffer A (**orange label**)	CM02001		4 mL	12 mL
	Buffer B (**indigo label**)	CM02005		4 mL	12 mL
	PBS buffer (**grey label**)	CM02013		4 mL	12 mL
	Filter Devices for Oligo (Only one part# will be supplied, 2 or 6 units)	CM03CD003A	CM53405.1	2	6
		CM03CD010A	CM53405.2 CM53405.3		
	Filter Devices for Antibody	CM03CD050A	Any	2	6
	Collection Tubes for Filter	CM03CT0		8	24
	Desalting Spin Column	CM03SG50		4	12
	Collection Tubes for Spin Column	CM03CT9		4	12
	1.5 mL Centrifuge Tubes	CM03CT2		1	3
User Supplied Material	IgG Antibody	N/A	NOT PROVIDED (**0.25–1 mg IgG** needed per reaction)		
	Amine Oligo (≥5 bases)	N/A	NOT PROVIDED (**4–15 nmol**, use Equation 1 (**Eq. 1**) to obtain the exact amount needed per reaction)		

Reaction Scale: The protocol is optimized for conjugating 1 mg of IgG antibody (**Ab**) with 15 nmol of oligo per reaction. If you have less than 1 mg of antibody, use Equation 1 (**Eq. 1**) below to obtain the amount (**Amt**) of oligo needed per reaction:

$$\textbf{Eq. 1}: Oligo\ Amt = Ab\ Amt\ in\ mg\_\_\_\_ \times 15 = \_\_\_\_ nmol$$

For steps in the protocol in which the buffer or reagent volume added changes according to the amount of Ab used, please use the corresponding Equation in the step to obtain the corrected volumes. Fill in the blank space in the step before proceeding to the operation.

Reaction Time: The preparation will typically take 6 h to complete. However, there are steps in the manual where you can take a break and come back later. Please read this manual carefully and finish the calculation if you have less than 1 mg of antibody before proceeding.

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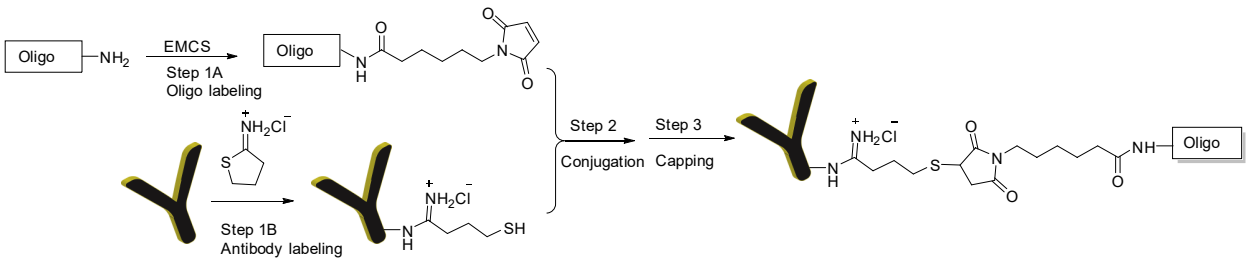
Safety Information

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

CellMosaic® has designed this personalized antibody-oligo conjugation kit to work with any antibody and an amine-modified oligo. The user supplies the amine-modified oligo (preferably a C6 amine modified oligo), which is readily available from many commercial oligo suppliers. Using the kit components, the customer converts the amine-oligo to a maleimide-oligo (Step 1A in **Scheme 1**). The customer also supplies their own antibody. Using the kit components, the customer converts some of the surface amines of the antibody to free thiol groups (Step 1B in **Scheme 1**), followed by reaction of the maleimide-modified oligo to generate the conjugates (Step 2 in **Scheme 1**). If there are any unreacted thiols on the antibody, they are subsequently capped (Step 3 in **Scheme 1**). The final conjugate is desalted, and buffer exchanged with PBS buffer.

An HPLC purified and lyophilized oligo is required for this conjugation. If the content of the amine oligo is >90%, the purity of the final conjugate will generally be >90% pure with an average 2 oligos per antibody. This purity is sufficient for ELISA, and a trace amount of oligo impurities and unreacted antibody will not affect the signal intensity too much. For complete removal of oligo impurities and unreacted antibody, gel filtration chromatographic purification is recommended.



Scheme 1: Synthetic route to antibody−oligo conjugate.

There are four reactions in this protocol (See **Scheme 2** for a detailed workflow). The oligo labeling with maleimide group (**Step 1A**) and antibody labeling with thiol groups (**Step 1B**) can be done simultaneously. However, keep in mind that maleimide-labeled oligo should be purified quickly once the reaction is done, and the purified maleimide-labeled oligo needs to be obtained prior to the purification of thiol-activated antibody. This sequence is important to ensure you have an activated antibody for the conjugation. Antibody-oligo conjugation is generally done within 1 h (**Step 2**), but you can go longer or leave it at 2−8°C overnight after the reaction. Capping is done within 30 minutes (**Step 3**) and the purification will take less than 10 minutes.

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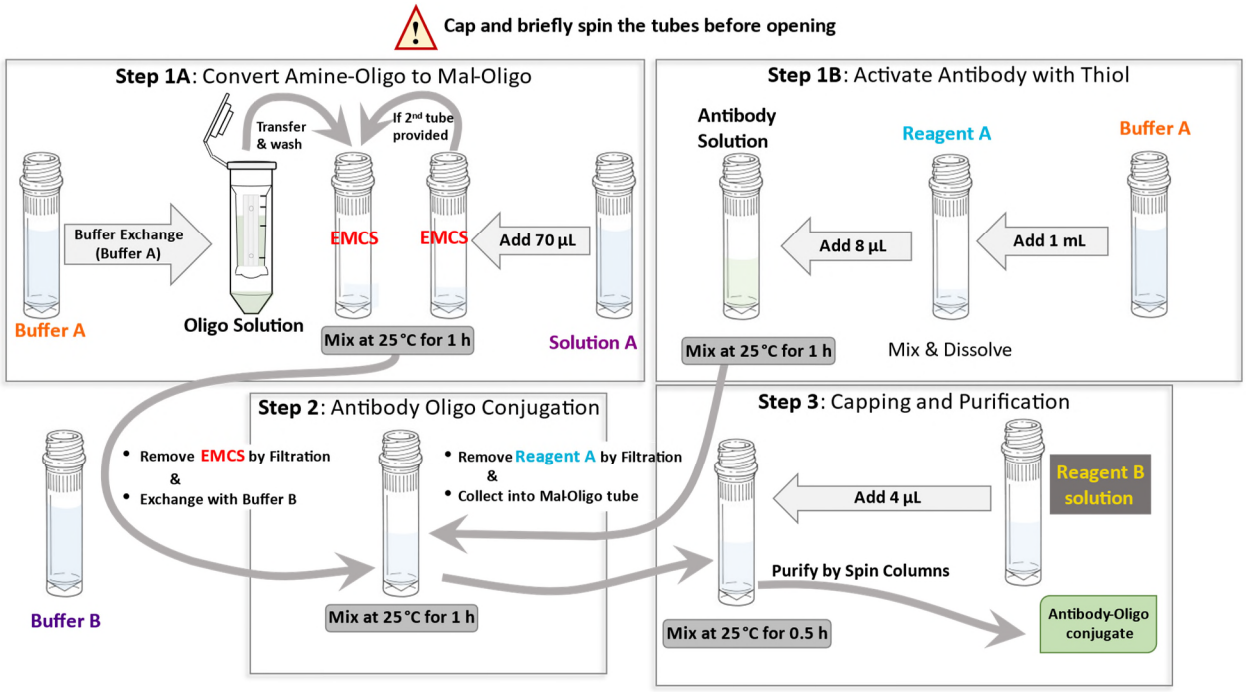
Key features of this antibody–oligo conjugation kit:

- Offers a convenient way to prepare antibody–oligo conjugate with heterobifunctional crosslinking reagents.
- Optimized precise loading with an average of two oligos per antibody for high performance.
- Preparation can be done in a day (less than 8 h) with various break points.
- ≥90% conjugate if the quality of the oligo is at par.
- All reagents and supplies were included for preparation and purification.
- Options to choose tailored services at CellMosaic after conjugation:
    - You can choose to send your conjugates to CellMosaic for HPLC analysis of the sample or complete removal of trace oligo impurities and unreacted antibody.

Support

A customer can request a recommendation for the conjugation if the oligo has some special features or solubility issues. CellMosaic also provides fee-based support services to customers who need help analyzing the final conjugates by HPLC and further purification to remove trace oligo impurities.

Protocol



Scheme 2. Schematic diagram of the workflow for preparing antibody–oligo conjugates starting with 1 mg of antibody.

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1. Lab Instrumentation Needed

- Vortex mixer, centrifuge (preferably refrigerated for example Eppendorf 5417R)
- Pipettes and tips
- Timer
- Incubator or shaker set at 25°C (room temperature between 20–27°C is acceptable)
- 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)

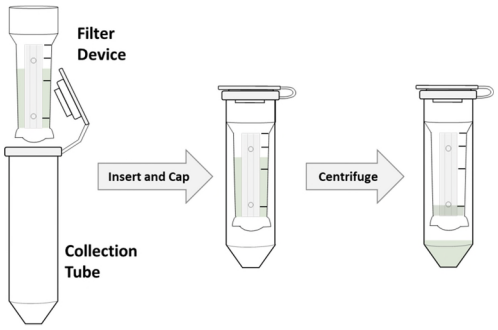
2. Preparation of Oligo Sample for Labeling

Items needed: Amine-Oligo (user supplied), Filter Device for Oligo (CM03CD003A or CM03CD010A), Collection Tubes for Filter (CM03CT0), Buffer A (CM02001, orange label), clean centrifuge tubes (not provided in the kit).

Total amount of oligo used for the conjugation is **4–15 nano-mole**.

A1. Insert a new Filter Device into one of the provided Collection Tubes. Perform the step based on the following conditions.

- ✓ If your oligo is supplied as a lyophilized solid, dissolve the oligo in deionized water to a concentration of 100 µM and then transfer up to 150 µL (15 nmol) or _____ µL (calculated from **Eq. 2**) of amine-oligo solution to the Filter Device. Add Buffer A (orange label) to make up the total volume to 500 µL and cap it.



- ✓ If your oligo is supplied as liquid, transfer up to **15 nano-mole** to the Filter Device directly. Add Buffer A to make up the total volume to 500 µL and cap it.

Calculation for less antibody:

Eq. 2: $Vol.of\ Oligo\ in\ Step\ \boldsymbol{A1} = Oligo\ Amt\ from\ \boldsymbol{Eq.1}$___ $\times \frac{\mu L}{nmol} =$ ____μL

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 (preferably cooled to 4°C) for 8 to 15 minutes to concentrate to < 100 µL. Spin time will depend on which Filter Devices are supplied in the kit.

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Catalog No# (1 rxn, 3 rxn)	Oligo Length	Spin Time	Typical Leftover Vol.
CM53405.1(x1, x3)	5-30 bases	15	80 µL
CM53405.2(x1, x3) CM53405.3(x1, x3)	≥31 bases	8	35 µL
Typical leftover volume is obtained using an Eppendorf 5417R and centrifuge 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 µL of Buffer A to the Filter Device. Spin the device at 14,000 x g for 8 to 15 minutes to concentrate to < 100 µL. Remove the assembled device from the centrifuge. Transfer the filtrate from the collection tube to a clean centrifuge tube (not provided). **Save the filtrate until the experiments are done.**

3. Convert Amine-Oligo to Maleimide-Oligo (Step 1A)

Items needed: Amine-Oligo from Step A5, Buffer A (CM02001, orange label), Solution A (CM02057, purple label), 1 or 2 tubes of EMCS (CM12107.1, red label).

B1. Briefly spin the tube containing EMCS (red label) before opening it.

B2. Spin Solution A (purple label) to ensure there is no liquid in the cap before opening it. Add 70 µL of Solution A to the EMCS tube. Vortex for 30 seconds to 1 minute to dissolve the reagent and then centrifuge to ensure no liquid is in the cap. **If two tubes of EMCS are supplied in Box 1 (Cat# CM53405.3 for labeling ≥60 bases oligo), transfer the mixture from the 1st EMCS tube to the 2nd EMCS tube. Repeat the mixing by vertexing the mixture for 30 seconds to 1 minute and then spin down.**

B3. Transfer the Amine-Oligo sample from the Filter Device from **Step A5** to the EMCS tube from **Step B2** by pipetting (preferably using a sterilized 100 µL pipette tip with filter). Make sure the pipette tip reaches the bottom of the Filter Device.

B4. Wash the Filter Device twice with 10 µL of Buffer A and transfer the wash to the same EMCS tube from **Step B2 (Note: Wash = add buffer, aspirate with pipette 2-3 times).**

B5. Spin the tube containing Buffer A (orange label) before opening it. Transfer 35 µL of Buffer A to the EMCS tube containing amine-oligo from **Step B4**.

B6. Vortex the solution for 30 seconds, and then centrifuge to ensure no liquid is in the cap. Mix at 25°C (RT between 20 to 27°C is acceptable) for exactly 1 h and move on to the purification steps (**Step E1**) immediately.

Start Time: _____ End Time:_____

Tip for mixing: You can use a nutator, shaker, vortex, or incubator shaker for mixing. If you are using end to end nutating, make sure your centrifuge is capped properly. If you don't

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have any of this equipment, you can let the centrifuge tube sit at the bench with manual mixing by pipetting every 20 minutes.
Time-saving tip: While waiting for the oligo labeling reaction to complete, move on to **steps C1–D3**. If the oligo labeling reaction is done before any of these steps, you can start purification (**steps E1–E7**) while performing **steps C1–D3** on the side.

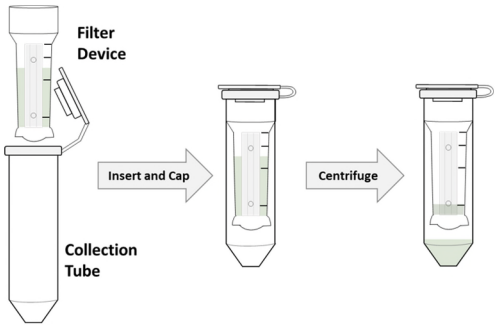
4. Preparation of Antibody Sample for Labeling

Items needed: Antibody (user supplied), Filter Devices for Antibody (CM03CD050A), Collection Tube for Filter (CM03CT0), Buffer A (CM02001, orange label), Clean Centrifuge Tubes (not provided in the kit).

The total amount of antibody used for the conjugation is 1 mg (protein content measured by UV). If you have less than 1 mg of antibody, use the equations in the protocols to obtain the corrected volumes to be added.

C1. Insert the **Filter Device for Antibody** (CM03CD050A) into one of the provided **Collection Tubes for Filter** (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 the Filter Device.
- ✓ If your antibody is supplied in < 500 µL buffer, transfer your antibody sample to the Filter Device directly. Add Buffer A to make up the total volume to 500 µL and cap it.
- ✓ If the volume of your antibody sample is >500 µL, add up to 500 µL of sample to the Filter Device. Repeat Step **C1-C4** until all of the antibody sample goes into the Filter Device. Move on to Step **C2**. Add Buffer A to make up the total volume to 500 µL for the last refill.



C2. Place the capped Filter Device into the centrifuge rotor, aligning the cap strap toward the center of the rotor, counterbalance with a similar device.

C3. Spin the Filter Device at 14,000 x g for 8 to 15 minutes (preferably cooled to 4°C) to concentrate to < 50 µL.

C4. 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.**

C5. Insert the Filter Device back into the collection tube. Add 400 µL of Buffer A into the Filter Device. Spin the device at 14,000 x g to concentrate to < 50 µL. Remove the assembled device

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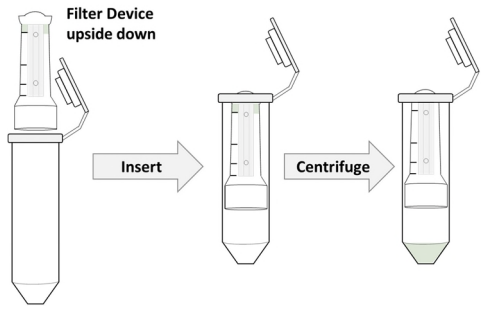
from the centrifuge. Transfer the filtrate from the collection tube to a clean centrifuge tube (not provided). **Save the filtrate until the experiments are done.**

C6. Repeat **Step C5** one time.

C7. To recover the antibody sample, place the Filter Device upside down in a clean Collection Tube. Place the Filter Device in the centrifuge rotor, aligning the cap strap toward the center of the rotor, counterbalance with a similar device. Spin for 2 minutes at 1,000 x *g* to transfer the antibody from the Filter Device to the Collection Tube.



C8. Wash the filter two times with 80 µL or _____µL Buffer A (calculated from **Eq. 3**) and transfer the wash to the Collection Tube from **Step C7**

(Note: Wash = Add buffer, aspirate with pipette 2-3 times.)

Calculation for less antibody (Ab):

Eq. 3: Buffer A $in\ Step\ C\mathbf{8} = Ab\ Amt\ in\ mg\_\_\_\_ \times 80 = \_\_\_\_\mu L$

C9. Vortex the combined antibody sample for 10 seconds and then centrifuge to ensure no liquid is in the cap.

HOLD Label the collection tube containing antibody from **Step C9** and place it in a 2−8°C refrigerator or an ice bucket. Wait until the oligo labeling is done (**Step B5**) before moving to **Step D1**.

5. Activate Antibody with Thiol (Step 1B, Continue from Step C9)

Items needed: Reagent A (CM12101, cyan label), Buffer A (CM02001, orange label).

D1. Briefly spin the tube containing Reagent A (cyan label). Add 1 mL of Buffer A to the tube with Reagent A. Vortex for 10−30 seconds to dissolve the reagent.

D2. Transfer 8 µL or _____µL (calculated from **Eq. 4**) of **Reagent A** solution from **Step D1** to the collection tube containing antibody solution from **Step C9**.

Calculation for less antibody:

Eq. 4: $\boldsymbol{Reagent\ A}\ solution\ transferred\ in\ \boldsymbol{Step\ D2}\ (\mu L) = Ab\ in\ mg\ \times 8 = \_\_\_\_\mu L$

D3. Vortex the solution for 30 seconds, and then centrifuge to ensure no liquid is in the cap. Mix the reaction mixture at 25°C (RT between 20 to 27°C is acceptable) for 1 hour.

Start Time: _____ End Time:_____

6. Purification of Maleimide Oligo to Remove Excess EMCS (Continue from Step B6)

Items needed: Oligo Labeling Solution from **Step B6**, Filter Device for Oligo (CM03CD003A or CM03CD010A), Collection Tube for Filter (CM03CT0), Buffer B (CM02005, indigo label), Clean Centrifuge Tubes (not provided in the kit).

E1. Insert one of the Filter Devices for Oligo (CM03CD003A or CM03CD010A) into one of the provided Collection Tubes for Filter. Transfer the EMCS-labeled oligo solution from **Step B6** into the Filter Device directly. Wash the oligo tube with 300 µL of Buffer B (indigo label) and transfer the wash to the Filter Device.

E2. Spin the Filter Device at 14,000 x *g* for 8 to 15 minutes (preferably cooled to 4°C) to concentrate to < 100 µL (refer to **Step A3** for spin time).

E3. Remove the assembled device from the centrifuge. Transfer the filtrate from the collection tube to a clean centrifuge tube (not provided). **Save the filtrate until the experiments are done.**

E4. Insert the Filter Device back into the collection tube. Add 400 µL of Buffer B to the Filter Device. Spin the device at 14,000 x *g* for 8 to 15 minutes to concentrate to < 100 µL.

E5. Repeat **E3** and **E4** two times.

E6. To recover the oligo sample, place the Filter Device upside down in a new collection tube. Spin for 2 minutes at 1,000 x *g* (preferably cooled to 4°C) to transfer the oligo sample from the Filter Device to the collection tube.

E7. Wash the filter once with 20 µL of Buffer B and transfer the wash to the collection tube.

(Note: Wash = Add buffer, aspirate with pipette 2-3 times.)

HOLD	Label the collection tube containing maleimide-oligo from **Step E7** and store it at ≤-20°C until the antibody sample is ready (**Step F6**).

7. Purification of Thiol Antibody to Remove Excess Reagent A and Conjugation with Oligo (Step 2)

Items needed: Filter Device for Antibody (CM03CD050A), Collection Tubes for Filter (CM03CT0), Buffer B (CM02005, indigo label), Maleimide-Oligo from **Step E7**, Clean Centrifuge Tubes (not provided in the kit), Thiol Antibody Solution from **Step D3**.

Steps F3 to **F7** are to be performed without any break. Reduced thiols tend to oxidize quickly. Make sure **Step E7** is completed prior to the following steps. Work quickly through **Steps F3-F7**.

F1. Insert one of the Filter Devices for Antibody (CM03CD050A) into one of the provided collection tubes. Spin the thiol-modified antibody from **Step D3** to ensure there is no liquid in the cap before opening it. Transfer the antibody solution into the Filter Device. Wash the

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antibody tube once with 200 µL of Buffer B (indigo label) and transfer the wash to the Filter Device. Add Buffer B to make up the total volume to 500 µL and cap it.

F2. Spin the Filter Device at 14,000 x *g* for 8 minutes (preferably cooled to 4°C) to concentrate to < 50 µL.

F3. Remove the assembled device from the centrifuge. Transfer the filtrate from the collection tube to a clean centrifuge tube (not provided). **Save the filtrate until the experiments are done.**

F4. Insert the Filter Device back into the collection tube. Add 400 µL of Buffer B to the Filter Device. Spin the device at 14,000 x *g* for 8 minutes to concentrate to **< 50 µL**. Remove the assembled device from the centrifuge. Transfer the filtrate from the collection tube to a clean centrifuge tube (not provided). **Save the filtrate until the experiments are done.**

F5. Repeat **Step F4** once.

F6. Take the collection tube containing maleimide-oligo from **Step E7** out of the freezer and place the Filter Device from **Step F5** upside down in this Collection Tube. Spin for 2 minutes at 1,000 x *g* (preferably cooled to 4°C) to transfer the thiol antibody from the Filter Device to the collection tube containing maleimide-oligo.

F7. Wash the filter once with 20 µL of Buffer B and transfer the wash to the collection tube.

(Note: Wash = Add buffer, aspirate with pipette 2-3 times.)

F8. Vortex the solution for 30 seconds, and then centrifuge to ensure no liquid is in the cap. Mix the reaction mixture at 25°C (RT between 20 to 27°C is acceptable) for 1 h.

Start Time: _____ End Time:_____

BREAK

Conjugation is usually done within 1 h. However, you can leave the reaction for a longer time or place it at 4°C overnight after 1 h at RT. If you have a lower amount of antibody (<0.5 mg) or oligo with a high number of nucleobases (≥40-mer), you can also let it go for an extended time (2–4 h).

8. Capping Unreacted Thiol Groups of Antibody (Step 3)

Items needed: Reagent B solution (CM12004.1, yellow label), Conjugate Solution from **Step F8**.

G1. Briefly spin the tube containing Reagent B solution (yellow label). Transfer 4 µL or ____ µL (calculated from **Eq. 5**) Reagent B solution to the reaction mixture from **Step F8**.

Calculation for less Antibody (Ab):

Eq. 5: $\textbf{Reagent B}\ solution\ added\ in\ \textbf{Step G1} = Ab\ in\ mg\_\_\_ \times 4 = \_\_\_\_\mu L$

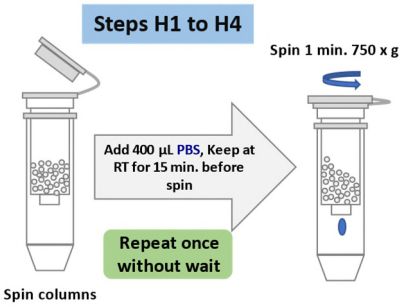
G2. Vortex the solution for 30 seconds, and then centrifuge to ensure no liquid is in the cap. Mix the reaction mixture at 25°C (RT between 20 to 27°C is acceptable) for 30 minutes.

Start Time: _____ End Time:_____

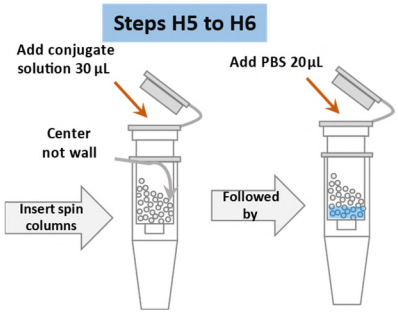
9. Purification to Remove Excess Reagent B and Unreacted Oligo (Step 3)

Items needed: Desalting Spin Column (CM03SG50), 1.5 mL Collection Tubes for Spin Column (CM03CT9), PBS buffer (CM02013, grey label), 1.5 mL Centrifuge Tube (CVM03CT2), Conjugate Solution from **Step G2**.

H1. Take out 4 desalting spin columns, remove the bottom red cap. Spin for 1 min at 750 x *g* before opening the top cap.
H2. Apply 400 µL of PBS buffer (grey label) to the top-center of the resin of each column. Let it remain at RT for 15 min to swell the resin.
H3. Spin for 1 min at 750 x *g* and discard the flow through. H
H4. Repeat **Steps H2–H3** once. Spin immediately after applying PBS without wait and discard the flow through.

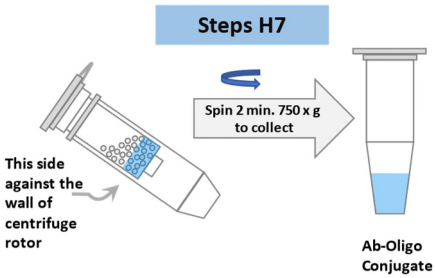


H5. Insert the spin columns into clean 1.5 mL collection tubes.
H6. Slowly apply up to 30 µL of conjugate solution from **Step G2** to the top-center of the resin of each spin column without disturbing the resin bed (4 x 30 µL). Then apply 20 µL of PBS buffer to the top-center of the resin of each spin column to make up the volume to 50 µL in each spin column.



> The resin may detach a little from the column to form a pillar with an unbalanced resin bed due to the centrifuge force. Make sure the sample and subsequent PBS buffer is applied slowly in the resin and not run down the sides of the resin bed. Wait for the conjugate solution to enter the resin before applying PBS buffer. Avoid touching the resin bed with the pipette tip.

H7. Rotate/align the spin column so that the higher resin bed side is against the wall of the centrifuge rotor and the lower resin bed faces the center of the centrifuge (same centrifuge force). Spin for 2 min at 750 x *g* to collect the fractions.



H8. Transfer and combine the fractions from the four collection tubes into the provided 1.5 mL centrifuge tube and cap it.

Antibody–Oligo is Ready for Your Experiment

You can assume 85% recovery for the antibody–oligo conjugates to estimate the concentration. The number of oligo molecules per antibody is ~2 on average.

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Other Considerations

1. Concentration Determination for IgG Antibody (Unlabeled)

The accuracy of the IgG amount is important for obtaining optimized oligo labeling in this protocol. The simplest assay method for determining IgG concentration in solution is to measure the absorbance at 280 nm (UV range) ($A_{1\ mg/mL}$ = 1.4).

If your antibody comes with a buffer that has no UV absorbance at 280 nm, you can measure the UV absorbance prior to starting an experiment.

$$Concentration\ (mg/mL)\ of\ IgG = \frac{(A280)}{1.4}$$

If your antibody comes with a buffer that has UV absorbance at 280 nm, you can determine the concentration in **Step C9** after exchanging it with **Buffer A** and assuming **95%** recovery of the IgG after buffer exchange. **Buffer A** does not contain any substances that will interfere with the UV measurement at 280 nm.

$$Concentration\ (mg/mL)\ of\ starting\ IgG = \frac{(A280)}{1.4 \times 0.95}$$

2. Concentration Determination for Oligo (Unlabeled)

The accuracy of the oligo amount is important for obtaining optimized oligo labeling in this protocol. The oligo manufacturer will usually supply the oligo in lyophilized form with the amount measured prior to lyophilization. This quantitation is usually sufficient for obtaining 2 oligos per antibody. For accuracy, you can re-measure the concentration of the oligo after re-suspending the oligo in deionized water in **Step A1**.

$$Concentration\ (M)\ of\ oligo\ before\ dilution = \frac{(A260) \times F}{L \times \varepsilon o}$$

A260: UV absorbance of oligo at 260 nm

L: UV cell path length (cm) - if you are using a 1 cm UV cell, you can dilute the oligo 1000-times to get a good reading

F: dilution factor

Ɛo: extinction coefficient of oligo

3. Concentration Determination for Antibody-Oligo Conjugate

To determine the concentration of your conjugate, dilute your antibody−oligo with 1x PBS buffer. Measure the UV absorbance of the antibody−oligo at 260 nm (A260) using a UV spectrometer and calculate the concentration using the following formula:

$$Concentration\ (\mu M) of\ the\ diluted\ sample = \frac{\mathrm{A260}\ \times 10^6}{\mathrm{L}\ \times (118100 + \mathrm{n} \times \varepsilon \mathrm{o})}$$

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$$Concentration\ (mg/mL)\ of\ the\ diluted\ sample = \frac{\mathrm{A260}\ \times (150000 + \mathrm{n} \times \mathrm{Mw(oligo)})}{\mathrm{L}\ \times (118100 + \mathrm{n} \times \mathrm{\varepsilon o})}$$

L: UV cell path length (cm) - if you are using a 1 cm UV cell, you can dilute the antibody–oligo 5 to 10-times to get a good reading

n: average number of oligo molecules per antibody

Ɛo: extinction coefficient of oligo

Extinction coefficient of IgG (MW of 150,000) at 260 nm is ~**118,100** $M^{-1}cm^{-1}$

Mw(oligo): molecular weight of oligo

4. Degree of Oligo Labeling (DOL) by UV

In this kit, the target DOL is 2. The DOL varies depending on the amine content of your oligo and the quantitation of oligo and antibody.

To estimate the DOL, you can obtain the UV absorbance ratio (R) of unlabeled oligo, unlabeled antibody, and your conjugate first.

$$R = {(A260)}/{(A280)}$$

Then use the following formula to calculate the estimated DOL:

$$DOL = \frac{210000 \times (Rc - Ra)}{\mathrm{\varepsilon o}\ (1 - Rc/Ro)}$$

Ɛo: extinction coefficient of oligo at 260 nm

Rc: R value of conjugate

Ro: R value of unlabeled oligo

Ra: R value of unlabeled antibody

Extinction coefficient of IgG (MW of 150,000) at 280 nm is 210,000 $M^{-1}cm^{-1}$

If you do not know the Ro and Ra value, please use the following formula to calculate the estimated DOL:

$$DOL = \frac{210000 \times (Rc - 0.5624)}{\mathrm{\varepsilon o}\ (1 - Rc/2)}$$

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5. Recommended Storage Conditions

Antibody–oligo conjugate is relatively stable at RT and can be stored at 2–8°C for several weeks without any decomposition. However, depending on the stability of your antibody, we recommend storing the conjugate at 2-8°C only short-term and use it as soon as possible. Some antibody–oligo conjugates may be lyophilized for long-term storage.

6. Characterization of Antibody–Oligo by HPLC

Antibody–oligo conjugate can easily be characterized by size-exclusion chromatography (SEC) and anion exchange chromatography (AEX) HPLC. SEC separates the molecules by apparent molecular weight (MW) or size in aqueous solution. The larger the MW of the conjugate, the earlier it elutes. By comparing the SEC profile of the unlabeled antibody, unlabeled oligo, and conjugate, you can check whether a conjugate formed, the heterogeneity of the labeling, and the percentage of unlabeled oligo and antibody. AEX separates the molecules based on the net surface negative charge (total charge). After conjugation, the total negative charge of the conjugates may be different and result in separation. It may be difficult to obtain good data with AEX HPLC without optimization. We recommend customers use SEC HPLC as the main method for conjugate analysis.

CellMosaic offers two SEC standards (Product #: CM92004 and CM92005) for our customers to use with any SEC column. The CM92004 product sheet contains all of the information and methodology needed to run an SEC HPLC analysis.

If you do not have access to an HPLC facility, you can send your sample to CellMosaic for analysis.

7. Sample Submission for HPLC Analysis

If you are submitting samples to CellMosaic for HPLC analysis, please follow these instructions:

1) Go online: https://www.cellmosaic.com/hplc-analysis/, select SEC HPLC Analysis (Product# AS0023) and/or AEX HPLC Analysis (Product# AS0026), choose the quantity (number of samples - bulk discounts are available for multiple samples), and submit the order. Alternatively, you can email info@cellmosaic.com for a quote and to place the order.
2) Dilute your un-conjugated antibody to 1 mg/mL in PBS buffer, and then transfer 50 µL of the diluted solution to a 500 µL micro-centrifuge tube. Label the vial properly.
3) Dilute your un-conjugated oligo to 10-20 µM in PBS buffer, and then transfer 50 µL of the diluted solution to a 500 µL micro-centrifuge tube. Label the vial properly.
4) Dilute your conjugate to 0.1–0.5 mg/mL in PBS buffer, and then transfer 50 µL of the diluted solution to a 500 µL micro-centrifuge tube. Label the vial properly.
5) Ship your samples with a cold pack for overnight delivery.

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

Antibody information: A therapeutic antibody (human IgG1 subtype)
Oligo information: HPLC-purified 18-mer oligo with 5' NH_2-C6 modification
Kit lot number: S331.S9.040721
Scale of the reaction: 1 mg of antibody
Specification of the final conjugates:

Calculated average DOL: 1.9	Unreacted antibodies: 9.3%
Unreacted oligo: ~0.5%	Conjugate purity: >90% (85% recovery)

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