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HRP-Oligo Conjugation Kit (CM53401x1 and CM53401x3) User Reference Guide

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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 oligomer, 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 are provided as necessary.

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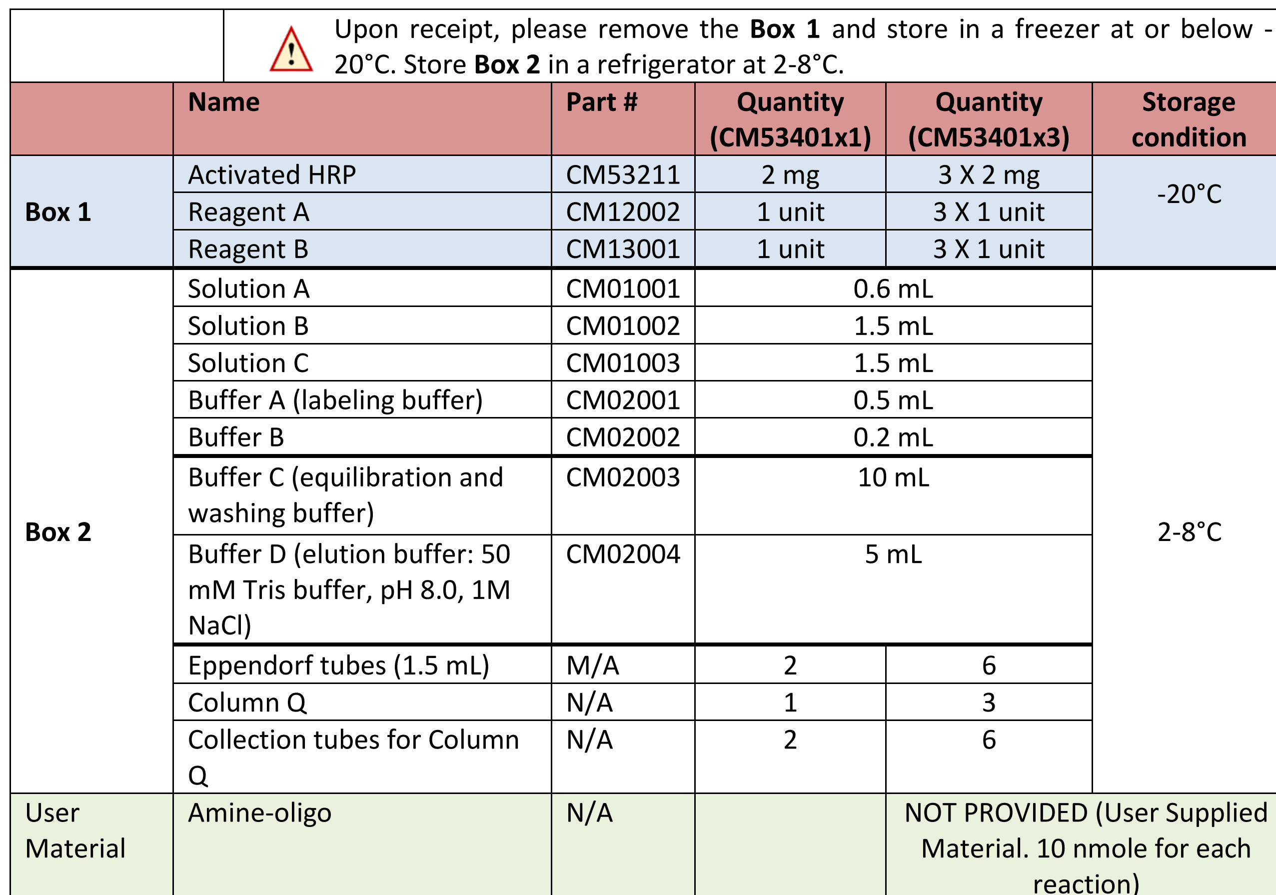
Kit Components

This kit provides materials to perform HRP labeling of one (CM53401x1) or three oligo samples (CM53401x3).



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

	Name	Part #	Quantity (CM53401x1)	Quantity (CM53401x3)	Storage condition
Box 1	Activated HRP	CM53211	2 mg	3 X 2 mg	-20°C
	Reagent A	CM12002	1 unit	3 X 1 unit	
	Reagent B	CM13001	1 unit	3 X 1 unit	
Box 2	Solution A	CM01001	0.6 mL		2-8°C
	Solution B	CM01002	1.5 mL		
	Solution C	CM01003	1.5 mL		
	Buffer A (labeling buffer)	CM02001	0.5 mL		
	Buffer B	CM02002	0.2 mL		
	Buffer C (equilibration and washing buffer)	CM02003	10 mL		
	Buffer D (elution buffer: 50 mM Tris buffer, pH 8.0, 1M NaCl)	CM02004	5 mL		
	Eppendorf tubes (1.5 mL)	M/A	2	6	
	Column Q	N/A	1	3	
	Collection tubes for Column Q	N/A	2	6	
User Material	Amine-oligo	N/A		NOT PROVIDED (User Supplied Material. 10 nmole 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

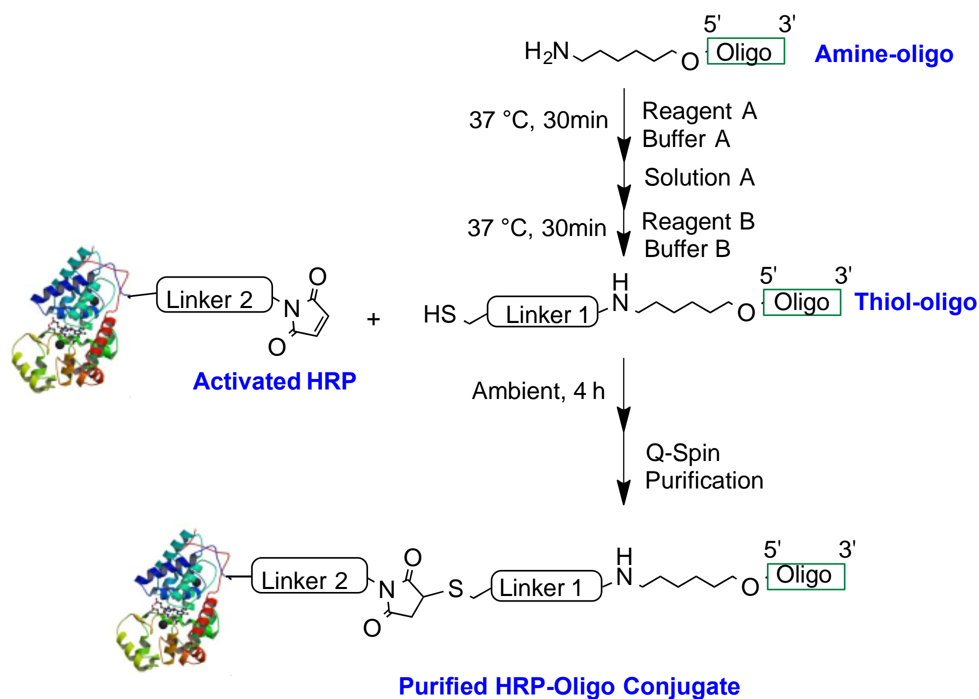
CellMosaic has designed this HRP-Oligo conjugate with linkers and conjugation chemistry that retain the activity of HRP. The same configuration and chemistry has been tested for HRP-Oligos with different oligo sequences in few diagnostic companies since 2011.

The kit is designed to work with amine-modified oligos. The user supplies the amine-modified oligos, which are readily available from many commercial oligo suppliers. Using the kit components, the user converts the amine-oligo to a thiol-oligo, followed by reaction of the thiol-oligo with activated HRP to

generate the HRP-oligo conjugates. A hydrophilic linker was chosen to enhance aqueous buffer solubility. The Q-column purification step typically provides the resulting HRP-oligo at greater than 80% purity.

Key features of this HRP-oligo conjugation kit:

- High quality maleimide activated HRP for the conjugation: >99% purity and >200 units/mg protein activity
- Optimal maleimide groups per HRP for single label of oligo: 1.2 for typical batch
- A single purification affords 85-90% of single labeled HRP-oligo conjugates
- Fast preparation: less than 2 h hands-on time
- Easy purification: less than 30 minutes
- Stable linkage with optimal PEG spacer to make sure there is no interference between oligo binding and HRP activation
- All reagents included, from preparation to purification



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Requirement for amine-oligo:
1. Preferably, the amine-oligo should be HPLC purified and lyophilized
2. Additional components of acid or other molecules containing primary or secondary amine groups and thiol should NOT be present

Potential interfering compounds for labeling and conjugation reactions:
a. *Thiols*: e.g., DTT and mercaptoethanol
b. *Primary and secondary amines*: e.g., ammonium acetate, ammonium bicarbonate, ammonium tartrate, ammonium citrate, ammonium phosphate, AMPD [2-amino-2-methyl-1,3 propanediol], aminoguanidine bicarbonate salt, ethanol amine, Gly-gly, Tris buffer, piperidine buffers.

Protocol

1. Lab Instrumentation Needed

- Vortex mixer, Centrifuge (preferably refrigerated)
- Pipettes and tips
- Timer
- Heat block or incubator set at 37 °C.
- -20°C freezer or ice bath
- Speed vacuum concentrator (optional)
- UV spectrophotometer (optional)

2. Convert Amine-Oligo to Thiol-Oligo (10 nmol Scale)

Items needed: Amine Oligo (user supplied), Reagent A (CM12002, white), Buffer A (CM02001, orange), Eppendorf Tube, Buffer B (CM02002, Oliver), Solution A (CM01001, yellow), Solution B (CM01002, violet), Solution C (CM01003, green), Reagent B (CM13001, blue).

A1. Re-suspend the amine-oligo in water to a concentration of 2 mM. Transfer 5 µl (10 nmol) of amine-oligo solution to a 1.5 mL **Eppendorf Tube**.

A2. Briefly spin the tube containing **Reagent A**. Make sure you can see a tiny droplet at the bottom of the tube. Add 20 µL of **Buffer A (labeling buffer)** 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.

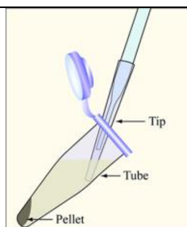
Tip for opening centrifuge tube after vortex: Always centrifuge the tube to make sure no liquid is in the cap.

A3. Transfer the entire **Reagent A** solution from **Step A2** to the 1.5 mL **Eppendorf Tube** containing 5 µL of amine-oligo solution from **Step A1**. Pipette the solution up and down in the tube three times to mix. Incubate at 37°C for 30 min.

A4. Remove the 1.5 mL **Eppendorf Tube** from **Step A3** from the incubator and add 2.5 µL of **Buffer B** and 100 µL of **Solution A**. Pipette the solution up and down three times in the tube to mix. Place on ice or at -20°C (freezer) for at least 20 min.

A5. Centrifuge the **Eppendorf Tube** from **Step A4** at 17,900 x g for 10 min (preferably at 4°C). Notice where the oligo pellet is. Carefully remove the supernatant (do not disturb the pellet).

Tip: Orient the hinge of the microcentrifuge tube outward in the centrifuge to assist in locating the nucleic acid pellet, which will then be located on the same side of the hinge.



A6. Add 200 µL of **Solution B** to the pellet. Invert the tube to mix. Spin briefly. Carefully remove the supernatant (do not disturb the pellet).

A7. Repeat **A6** one more time. Remove as much residual liquid as possible.

Tip: Keep the supernatant in case the pellet is dislodged and needs to be recovered.

A8. Air dry for 5 to 10 minutes or briefly vacuum dry the pellet using a speed vacuum concentrator. Make sure there is no liquid droplet in the **Eppendorf Tube**.

A9. Re-suspend the pellet with 25 µL of **Solution C**.

A10. Centrifuge the tube containing **Reagent B** to spin down the solid**.** Make sure you can see a small amount of solid at the bottom of the tube. Add 25 µL of **Buffer A** to the tube. Vortex for 30 second to 1 minute to dissolve the reagent.

A11. Transfer the entire **Reagent B** solution from **Step A10** to the tube containing oligo from **Step A9**. Pipette the solution up and down in the tube three times to mix. Incubate at 37°C for 30 min.

A12 (Optional). To ensure the correct amount of oligo is used for the next step, dilute 2 µL of the oligo from **Step A11** with 398 µL of water. Measure the OD260 using a UV spectrometer and determine the concentration using the extinction coefficient of the oligo. The final concentration should be around 1 µM. Calculate the volume needed for 5 nmol of oligo for the next step.

Alternatively, you can assume 100% recovery, use 25 µL of the solution from **Step A11** for the next step.

Thiol-oligo is Ready for Conjugation

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3. HRP Conjugation with Oligo

Items needed: Activated HRP (CM53211, red), Activated Oligo from Step A11, Solution C (CM01003, green).

B1. Add 200 µL of **Solution C** to a tube containing **Activated HRP**. Vortex for 30 seconds to 1 minute to dissolve the HRP.
B2. Add the activated oligo (**25 µL or more based on concentration from Step A12**, ~5 nmol) from **Step A11** to the Activated HRP from **Step B1**. Pipette the solution up and down in the tube three times to mix. Incubate at room temperature **in the dark** for at least 4 hours, preferably overnight.

4. Purification to Remove Excess HRP

Items needed: Buffer C (CM02003, Lime),Column Q, Buffer D (CM02004, navy), Collection Tubes

C1, Sample Preparation: When **Step B2** is complete, add 175 µL of **Buffer C** (equilibration and washing buffer). Pipette the solution up and down in the tube three times to mix.

C2, Column Equilibration: Add 400 µL of **Buffer C** to a Column Q and centrifuge at 2000 x g for 2 minutes. Discard the flow-through. Repeat this step one more time.

C3, Sample Application: Add sample solution from **Step C1** to the equilibrated Column Q in a maximum volume of 400 µL. Centrifuge at 2000 x g for 5 minutes. Set aside the flow-through.

Tip: Pay attention to the color of Column Q; if the conjugation reaction is successful, the top of Column Q will be light brown. **If the top of the column Q is colorless, please store your remaining buffers/solvents/column Q inside the 4 °C refrigerator and contact CellMosaic.**

C4, Washing: Add 400 µL of **Buffer C** to Column Q and centrifuge at 2000 x g for 2 minutes. Repeat two more times. Make sure the final wash is colorless. Discard the flow-through.

C5, Elution: Place Column Q on a clean **Collection Tube**. Add 400 µL of **Buffer D** (elution buffer) to the column. Centrifuge at 2000 x g for 2 minutes. Collect the flow-through and label it as elution (**E1**).

Tip for elution volume: **E1** contains the majority of your purified HRP-oligo conjugates. However, conjugating longer oligos may require more volume to elute. Pay attention to the color of Column Q; if it is still brown-colored, repeat **Step C5** to elute and collect more fractions.

HRP-Oligo is Ready for Your Experiment

Tip: The approximate concentration of **E1** is 6 µM in 400 µL of 50 mM Tris buffer, pH 8.0, 1 M NaCl. Elution can be diluted (for lower concentration) or a desalting column (not included in kit) can be used to exchange the buffer.

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Other Considerations

1. Concentration Determination

To determine the concentration, dilute your HRP-oligo from **Step C5** with 1 x PBS buffer. Measure the UV Absorbance of HRP-oligo at 403 nm (A_{403}) using a UV spectrometer and calculate the concentration based on the following formula:

Concentration (µM) of diluted sample = (A_{403}) * 10 / (L x 1.02)

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

2. MW Calculation

Calculation of the MW of the conjugate:

Mw(conjugate) = n x Mw(oligo) + Mw(HRP) + 486

n: average molar ratio of oligo per HRP. Use **1.0** if you don't have the SEC profile of your conjugates.

3. Recommended Storage Conditions

For long-term storage, HRP-oligo conjugates can be lyophilized and stored as lyophilized powder at -20°C for 1 year.

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

Oligo information: HPLC-purified 18mer oligo with 5' NH_2-C6 modification

Kit Lot number: 5500.S7.101717

Figure 1: Size-exclusion HPLC analysis of the HRP and oligo reaction mixture (before purification).

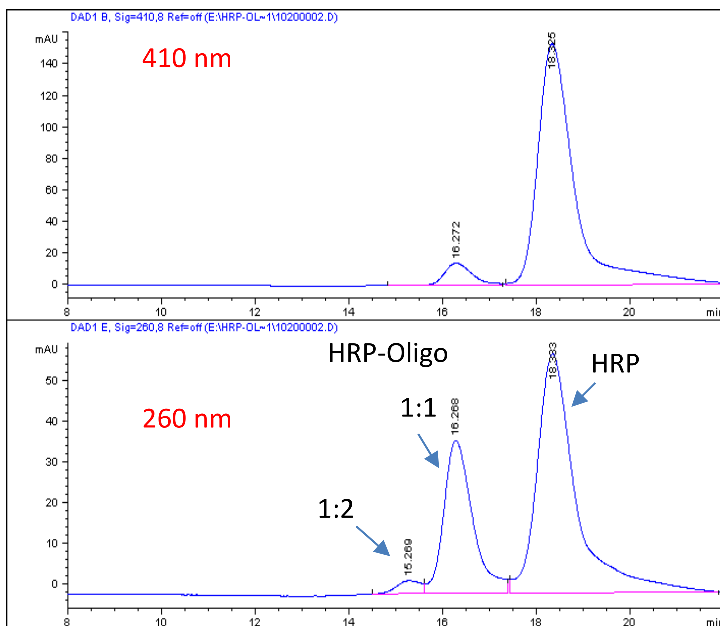

Figure 2: Size-exclusion HPLC analysis of first elution (E1) from Column Q (after purification).

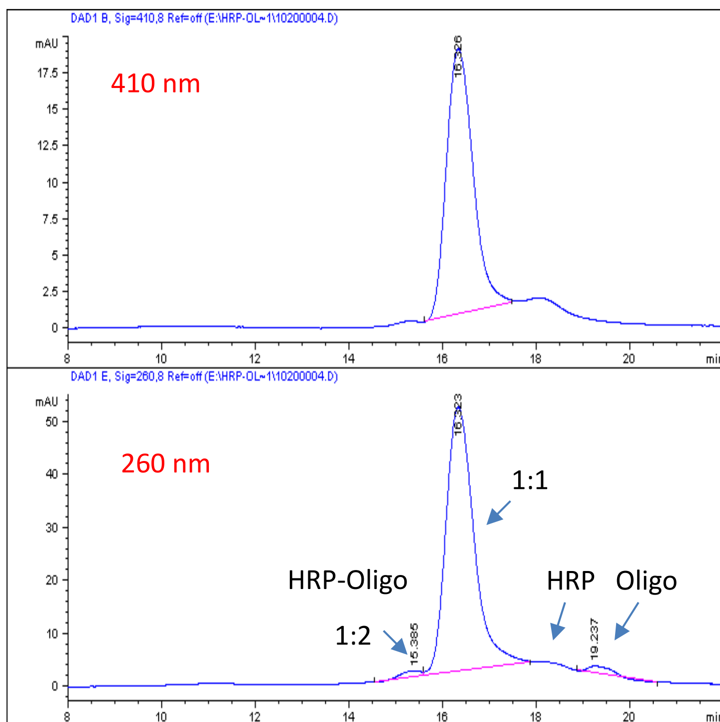

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Table 1: Summary results

Sample	Volume (μL)	% free HRP	% free oligo	% oligo/HRP (2:1)	% oligo/HRP (1:1)
E1 (first elution)	400	~4	~5	~1	90
Recovery: ~50% of the conjugates from 5 nmol of starting oligo.					