

# HRP-Oligo Conjugation Kit (Thiol Oligo) (CM53402x1 and CM53402x3) 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 (CM53402x1) or three oligo samples (CM53402x3).

 Upon receipt, please remove the <b>Box 1</b> and store in a freezer at or below -20°C. Store <b>Box 2</b> in a refrigerator at 2-8°C.					
	Name	Part #	Quantity (CM53401x1)	Quantity (CM53401x3)	Storage condition
<b>Box 1</b>	Activated HRP	CM53211	2 mg	3 X 2 mg	-20°C
	Reducing Reagent	CM13001	1 unit	3 X 1 unit	
<b>Box 2</b>	Solution A	CM01003	1.5 mL		2-8°C
	Reducing Buffer	CM02001	0.5 mL		
	Equilibration Buffer	CM02003	10 mL		
	Elution buffer: 50 mM Tris buffer, pH 8.0, 1M NaCl	CM02004	5 mL		
	Column Q	N/A	1	3	
	Collection tubes for Column Q	N/A	2	6	
User Material	Disulfide-oligo	N/A		NOT PROVIDED (User Supplied Material. 5 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 a protected thiol-modified oligos (disulfide form). The user supplies the thiol-modified oligos containing 3' C6 disulfide linker or 5' C6 disulfide linker, which are readily available from many commercial oligo suppliers. Using the kit components, the user reduces the disulfide bond, followed by reaction of the thiol-oligo with activated HRP to generate the HRP-oligo conjugates. The Q-column purification step typically provides the resulting HRP-oligo at greater than 85% purity.

Key features of this HRP-oligo conjugation kit:

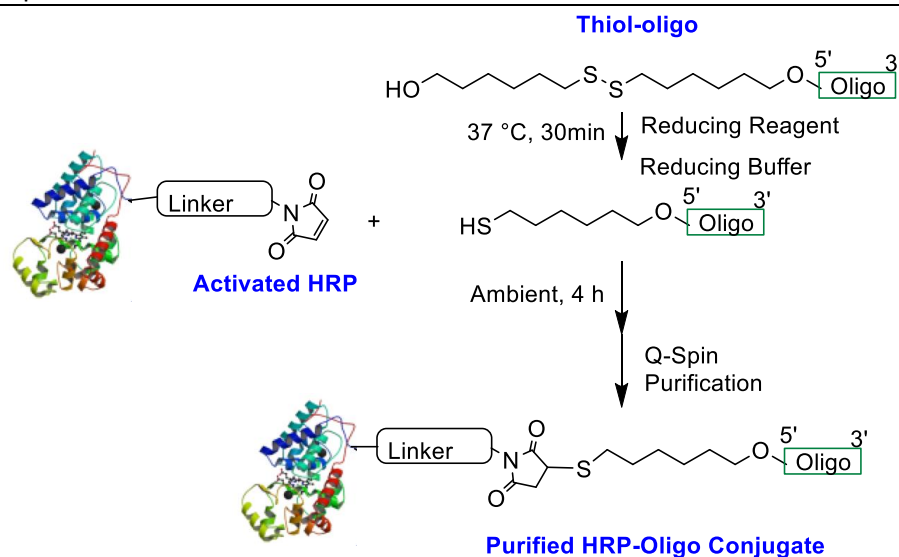
- High quality maleimide activated HRP for the conjugation: >99% purity and >200 units/mg protein activity
- Optimal maleimide groups per HRP for single label of oligo: 1.2 for typical batch

- A single purification affords 85-90% of single labeled HRP-oligo conjugates
- Fast preparation: less than 1 h hands-on time
- Easy purification: less than 30 minutes
- All reagents included, from preparation to purification

**Requirement for thiol-oligo:**

1. The oligo is modified with 3' C6 disulfide linker or 5' C6 disulfide linker
2. Preferably, the disulfide-oligo should be HPLC purified and lyophilized

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



## 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. Reducing the Disulfide Group of Oligo (5 nmol Scale)

Items needed: Disulfide-oligo (user supplied), Reducing Buffer (CM02001, orange), Reducing Reagent (CM13001, blue).

**A1.** Re-suspend the disulfide-oligo in water to a concentration of 1 mM.

**A2.** Centrifuge the tube containing **Reducing Reagent** to spin down the solid. Make sure you can see a small amount of solid at the bottom of the tube. Add 25  $\mu$ L of **Reducing Buffer** to the tube. Vortex for 30 second to 1 minute to dissolve the reagent.

**A3.** Transfer 5  $\mu$ L (5 nmol) of disulfide-oligo solution to the tube containing **Reducing Reagent** from **Step A2**. Pipette the solution up and down in the tube three times to mix. Incubate at 37°C for 30 min.

### Thiol-oligo is Ready for Conjugation

## 3. HRP Conjugation with Oligo

Items needed: Activated HRP (CM53211, red), Activated Oligo from Step A3, Solution A (CM01003, green).

**B1.** Add 200  $\mu$ L of **Solution A** to a tube containing **Activated HRP**. Vortex for 30 seconds to 1 minute to dissolve the HRP.

**B2.** Add the activated oligo from **Step A3** 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: Equilibration Buffer (CM02003, Lime), Column Q, Elution Buffer (CM02004, navy), Collection Tubes

**C1, Sample Preparation:** When **Step B2** is complete, add 150  $\mu$ L of **Equilibration Buffer**. Pipette the solution up and down in the tube three times to mix.

**C2, Column Equilibration:** Add 400  $\mu$ L of **Equilibration Buffer** 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  $\mu$ L. 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 and column Q inside the 4 °C refrigerator and contact CellMosaic immediately.**

**C4, Washing:** Add 400  $\mu$ L of **Equilibration Buffer** 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  $\mu$ L of **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  $\mu$ M in 400  $\mu$ L 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.

## 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:

$$\text{Concentration } (\mu\text{M}) = (A_{403}) * 10 / (L * 1.02)$$

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:

$$\text{Mw(conjugate)} = n * \text{Mw(oligo)} + \text{Mw(HRP)} + 267$$

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

### 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.