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PerKit™ HRP-Peptide Conjugation Kit (CM32401x1 and CM32401x3) 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 HRP labeling of one (CM32401x1) or three peptide samples (CM32401x3).

Table 1: Components and storage temperatures for PerKit™ HRP−Peptide conjugation kit.

- 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 #	Quantity (x1)	Quantity (x3)
Box 1 (≤-20°C)	C6 Maleimide Activated HRP (**red label**)	CM53214	1 unit	3 units
Box 2 (2−8°C)	Solution A (green label)	CM01003	1.5 mL	3 mL
	Solution B (purple label)	CM01007	0.5 mL	0.5 mL
	1xPBS buffer (grey label)	CM02013	6 mL	20 mL
	Centrifugal Filter Devices	CM03CD030A	2	6
	Collection Tubes	CM03CT0	4	12
	1.5 mL Centrifuge Tube(s)	CM03CT2	1	3
	0.5 mL Centrifuge Tube(s)	CM03CT1	1	3
User Supplied Material	Cys-peptide	N/A	NOT PROVIDED (User Supplied Material. 0.5 µmol 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 Safety Data Sheets (SDS) available at www.cellmosaic.com before you store, handle, or use any of the materials.

Labeling Chemistry

CellMosaic® has designed this personalized conjugation kit to work with any peptide containing a reactive Cys group. Using the kit components, the customer prepares the HRP-peptide conjugate by reacting their peptide (customer supplied) with activated HRP. One-step purification typically removes most of the unreacted peptides.

HRP contains two available primary Ɛ-amine groups for modification. The maleimide-activated HRP via surface amine has limited maleimides (average 1-2 maleimides per HRP) and is suitable for single labeling. Depending on the activity of your peptide, some unreacted HRP may be present in the final mixture. For typical ELISA and other solid-based immunoassays, the unreacted HRP can be washed away after peptide binding. For other applications that require complete removal of free HRP, column purification may be used to remove any unreacted HRP.

This kit provides materials to label one to three peptides. Total amount of activated HRP included in a reaction: 2 mg.

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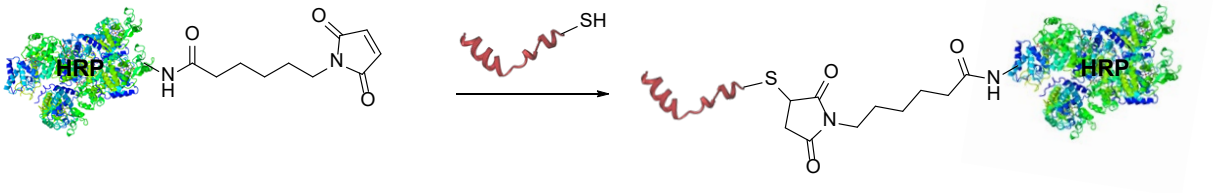
Key features of this HRP-peptide conjugation kit:

- High quality maleimide-activated HRP for conjugation: >99% purity and >200 units/mg protein activity.
- Optimal maleimide groups per HRP for single-labeled peptide: 1.5 for typical batch.
- A single purification removes the majority of unreacted peptides.
- Fast preparation: less than 1 h hands-on time.
- Options to choose tailored services at CellMosaic:
    - Prior to conjugation, you can supply your peptide information when you place your order and CellMosaic will give recommendations for the conjugation if your peptide has special features.
    - After conjugation, you can choose to send your sample to CellMosaic for HPLC analysis of the conjugates or HPLC purification to removal trace amounts of unreacted peptides and HRP.



Scheme 1. Synthetic route to HRP–Peptide conjugate.

Requirement for Cys-peptide:

1. Amount: 0.5 µmol
2. HPLC purified and lyophilized: please ensure no reducing reagents, such as DTT, are present
3. The Cys-peptide should be stored at -80°C
4. HPLC purity: >85% for *N*-terminal Cys-peptide and >90% for *C*-terminal Cys-peptide

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

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Protocol

1. Lab Instrumentation Needed

- Vortex mixer, centrifuge (preferably refrigerated, 14,000 g capable), mini-centrifuge
- Pipettes and tips
- Timer
- Incubator or shaker set at 25°C or RT
- Balance
- Personal protection equipment (lab coat, safety glasses, and chemical-resistant nitrile gloves)

2. HRP Conjugation with Peptide

Items needed: Cys-peptide (user supplied), C6 Maleimide Activated HRP (CM53214, red label), Solution A (CM01003, green label), Solution B (CM01007, purple label), 0.5 mL Centrifuge Tube (CM03CT1).

The following steps (A1-A4) are to be performed without any break. The free thiol of peptides tends to oxidize quickly once in solution. Similarly, maleimide-activated HRP is not very stable in solution for a prolonged period of time.

A1. Weigh 0.5 µmol of **Cys-peptide** into a **0.5 mL Centrifuge tube**. Note, the peptide is statically charged. Use the tip of a glass Pasteur pipet to weigh the peptide if possible. It may be difficult to obtain the exact weight. Any amount between 0.5 µmol and 0.8 µmol (160%) is acceptable (no need to adjust the volume of the dissolving solution).

Calculation for 0.5 µmol:

Amount of Cys-peptide (mg) = Molecular weight of Cys-peptide x 0.0005

A2. Add 400 µL of **Solution A** (green label) to a tube containing **C6 Maleimide Activated HRP** (red label). Vortex for 30 seconds to 1 minute to dissolve the HRP.
A3. Add 25 µL of **Solution B** (purple label) to the centrifuge tube containing Cys-peptide from **Step A1.** Vortex for 30 seconds and centrifuge the tube to get all the liquid down to the bottom. Open the cap and add 75 µL of **Solution A** (green label) to the tube. Vortex for 30 seconds or sonicate for a few minutes to ensure all the solid is dissolved. Discard any unused **Solution B** as hazardous chemical waste **when the experiments are done**.

Tip for solubility check (Step A3): It may take a while for your peptide to fully dissolve. In general, most of the peptide should be able to dissolve in this mixed solution system. Check the bottom of the micro-centrifuge tube to ensure the solution is clear and free of any solid residue. If some solid remains after a few minutes, centrifuge the tube, and pipette the supernatant for the next step.

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

A4. Transfer the entire solution from **Step A3** to the tube containing **Activated HRP** from **Step A2**. Pipette the solution up and down in the tube three times to mix. Incubate at RT for 2 hours.

Start Time: _____ End Time: _____

3. Purification to Remove Excess Peptide

Items needed: Reaction mixture from Step A4, 1xPBS buffer (CM02013, grey label), 2 Centrifugal Filter Devices (CM03CD030A), 4 Collection Tubes for Filter (CM03CT0), 1.5 mL Centrifuge Tube (CM03CT2).

B1. Place the centrifuge tube containing the **Reaction mixture from Step A4** into the centrifuge rotor and counterbalance with a similar device. Spin the **Centrifuge tube** at 10,000 x g for 2 minutes.

B2. Transfer and divide the supernatant from **Step B1** into two **Centrifuge Filter Devices**. Add 250 µL of **PBS Buffer** to make up the total volume to 500 µL in each filter device and cap it.

B3. Place the capped **Filter Devices** into the centrifuge rotor, aligning the cap straps toward the center of the rotor. Spin the **Filter Devices** at 14,000 x g for 8 minutes.

B4. Remove the **Filter Devices** from the centrifuge. **Save the filtrate until the experiments are done.**

B5. Add 400 µL of **PBS Buffer** to make the total volume 500 µL. Place the two capped **Filter Devices** into the centrifuge rotor, aligning the cap straps toward the center of the rotor. Spin the **Filter Devices** at 14,000 x g for 8 minutes.

B6. If the MW of your peptide is <3000 Da, repeat **Step B5** two times.

If the MW of your peptide is between 3000 and 5000 Da, repeat **Step B5** three times.

If the MW of your peptide is >5000 Da, repeat **Step B5** four times.

B7. To recover the conjugates, place the **Filter Device** upside down in a clean **Collection Tube**. Place it in the centrifuge, aligning the open cap towards the center of the rotor. Spin for 2 minutes at 1,000 x g to transfer the conjugates from the **Filter Device** to the **Collection Tube.**

B8. Transfer the conjugates from the two **Collection Tubes** to a **1.5 mL Centrifuge Tube**.

B9. Rinse each **Collection Tube** with 50 µL of **PBS Buffer** and transfer the entire contents to the **1.5 mL Centrifuge Tube** from **Step B8**.

B10. Add 100 µL of **PBS Buffer** to each **Filter Device** to rinse. Stir it gently with a pipet tip, then transfer the entire contents to the **1.5 mL Centrifuge Tube** from **Step B9**. Add **PBS Buffer** to make the total volume of the sample 400 µL and cap it (use the pipetman to measure the total volume).

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B11. Vortex the combined protein sample for 30 seconds and then centrifuge to ensure no liquid is in the cap.

HRP-Peptide is Ready for Your Experiment

Tip: The approximate concentration of **the HRP-peptide conjugate** is 100 µM in 400 µL of PBS buffer (4 mg/mL, assuming 80% recovery). You can also determine the concentration using a UV/Vis spectrophotometer.

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Other Considerations

1. Concentration Determination

To determine the concentration, dilute your HRP-peptide from **Step B11** with 1x PBS buffer. Measure the UV absorbance of the HRP-peptide at 403 nm (A_{403}) using a UV spectrometer and calculate the concentration based on the following formula:

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

Where L is the UV cell path length (cm). If you are using a 1 cm UV cell, you can dilute HRP-peptide 20-40 times to get a good reading.

2. MW Calculation

Calculation of the MW of the conjugate:

MW(conjugate) = n x MW(peptide) + 42800

Where n is the average molar ratio of peptide per HRP. Use 1.0 if you do not have the SEC profile of your conjugates.

3. Recommended Storage Conditions

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