UltraTag™ Hapten Labeling Kit Protocol

The UltraTag Hapten Labeling Kits efficiently conjugate 50 µg of antibody in 50 µL with one of four available hapten tags in less than an hour, with ~100% tagging, >80% recovery. Then, the tagged antibody can be used in any immunoassay and subsequently detected by the corresponding α-hapten antibody. Up to four antibodies tagged with the four different haptens can be used together and detected independently. The UltraTag technology does not interfere with other methods, such as conventional detection, and is compatible with biotin or digoxigenin detection, to allow independent analysis of up to six antigens.

The UltraTag Hapten Labeling Kit modification protocol requires three steps:

1. Buffer exchange 50 µg carrier-free antibody into Buffer A
2. Add buffer exchanged antibody to Linker A, incubate, and desalt
3. Add linker-modified antibody to hapten, incubate, and desalt

The only equipment required are a 100 µL pipette and a microcentrifuge.

This protocol can be downloaded from cellidx.com/products/ultratag/multiplex-labeling-kit

Kit Contents

Section	Row	Description
A	1	BLUE-capped desalting column to buffer exchange antibody with collection tube
	2	Antibody-linker reagent for modification of 50 µg IgG @ 0.9–1.1 mg/mL
	3	GREEN-capped desalting column to buffer exchange linker modified antibody with collection tube
	4	UltraTag Reagent
B	1	Link buffer
	2	RED-capped desalting column to buffer exchange tag-antibody conjugate into PBS with collection tube
	3	Rabbit α-UltraTag antibody-fluor, 15 µg @ 0.5 mg/mL
	4	Intentionally left empty

Antibody Preparation & Modification

1. **Desalting Column Preparation**: Break off bottom of BLUE-capped column, loosen the BLUE cap, and place column in collection tube, then place in microcentrifuge and spin for 3 minutes @ 1500 g. **Discard** flow-through and collection tube.
2. Add your antibody solution (50 µg @ 1 mg/mL) directly to the BLUE-capped column, replace BLUE cap loosely on column, insert in a new collection tube, and centrifuge for 3 minutes @ 1500 g.
3. Add flow-through from BLUE-capped column to Antibody Linker Reagent, pipette several times to mix, lightly vortex, and incubate for 1 hour @ room temperature.
4. **Desalting Column Preparation**: Break off bottom of GREEN-capped column, loosen the GREEN cap, and place column in collection tube, then place in microcentrifuge and spin for 3 minutes @ 1500 g. **Discard** flow-through and collection tube.
5. Add linker-modified antibody solution from Step 4 to GREEN-capped column, replace GREEN cap loosely on column, insert in a new collection tube, and centrifuge for 3 minutes @ 1500 g.
6. Add flow-through from GREEN-capped column collection tube from Step 5 to UltraTag labeling reagent tube, pipette several times to mix, and lightly vortex. Immediately continue to Step 7.

UltraTag Labeling & Purification

7. Add 7.5 µL of Link Buffer directly to the UltraTag labeling reagent tube from Step 7 pipette several times to mix, lightly vortex, and incubate for 1 hour @ room temperature.
8. UltraTag-conjugated Antibody Purification: Break off bottom of RED-capped column and loosen the RED cap, place column in collection tube, then place in microcentrifuge and spin for 3 minutes @1500 g. **Discard** flow through and collection tube.
9. Transfer the UltraTag-antibody conjugation mixture from Step 7 to the RED-capped column and centrifuge for 3 minutes @1500 g.
10. Transfer the conjugate to a final storage tube of your choice. The conjugate yield of this process is ~70–80%. Therefore, the final concentration of antibody will be ~0.7–0.8 mg/mL.

Staining

Pre-staining preparation:

1. Dilute the fluor-labeled antibodies with antibody diluent to **5 µg/mL**.
2. Recommended concentration for staining with UltraTag-antibody conjugate is **5 µg/mL**.
3. Recommended concentration for staining with fluor-labeled anti-UltraTag antibody and fluor-labeled secondary antibody is **5 µg/mL**.

Use your preferred primary antibody/fluor-labeled anti-species antibody antigen retrieval and staining procedure incubating for 1 hour with a cocktail of UltraTag primaries followed by incubation with a cocktail of fluor-labeled anti-tag antibodies.

Antigen Retrieval Method	Buffer recommended	Antigen Retrieval Time
Manual: pressure cooker	Citrate pH 6	15 minutes
Manual: PT Module	Dewax and HIER Buffer L (TA-100-DHBL)	5 minutes
BOND RX	ER2	20 minutes

Imaging

The table below presents the suggested exposure times and fluorophore properties of respective hapten/anti-Tag antibody pairs. Exposure times may vary depending on instrument used.

Anti-Tag Probe	Fluor	Absorbance (nm)	Emission (nm)	Extinction Coefficient (M-1cm-1)	Recommended Exposure Time (ms)
CH-015	CL-490	491	515	73,000	100-300
CH-014	CL-550	550	575	150,000	100-300
CH-021	CL-650	655	676	250,000	200-400
CH-019	CL-750	759	780	240,000	400-800

Note: Do NOT use VectorShield (Vectorlabs Catalog Number: H-1200).