UltraTag™ Multiplex Labeling Kit Protocol — Fluorescent

The UltraTag Multiplex Labeling Kits efficiently conjugate 50 µg of antibody in 50 µL with one of four available Tags in two hours, with highly efficient tagging, and >80% recovery. The tagged antibody can be used in any immunoassay and subsequently detected by the corresponding anti-Tag antibody. Up to four antibodies tagged with four different UltraTag Multiplex Labeling Kits can be used together and detected independently in a multiplex staining panel. To assemble a 4-plex panel, select the three individual UltraTag kits labeled with CL490, CL550, and CL650 fluors. Then select either the UltraTag kit labeled with CL595 or CL750 depending on whether your microscope or scanner can detect CL595 or CL750. See below:

Cat #	Tag	Anti-Tag Antibody–Fluor
UTFK-490	UT015	Anti-UT015–CL490
UTFK-550	UT016	Anti-UT016–CL550
UTFK-650	UT021	Anti-UT021–CL650
Choose **UTFK-595**	UT019	Anti-UT019–CL595
OR **UTFK-750**	UT019	Anti-UT019–CL750

The UltraTag technology does not interfere with other methods, such as conventional detection, and is compatible with biotin and digoxygenin detection, to allow independent analysis of up to six antigens.

The UltraTag Labeling Kit modification protocol is summarized below:

1. Buffer exchange 50 µg carrier-free antibody into Mod Buffer.
2. Add buffer exchanged antibody to Antibody Linker.
3. Add linker-modified antibody to UltraTag Reagent tube, incubate, and desalt.

The only equipment required are a 200µL pipette and a microcentrifuge.

This protocol can be downloaded from cellidx.com/documents/protocols/UltraTag-Multiplex-Labeling-Kit-Protocol-Fluorescent.pdf

Before Starting

- Ensure your antibodies for labeling are at 0.9-1.1mg/mL in buffer free of extraneous proteins such as BSA, serum, or gelatin. Antibody formulations containing azide, trehalose, or other sugars, as well as any buffer containing tris, are acceptable.
- Select antibodies that work well in your desired application. Antibodies with strong binding profiles are usually good candidates. We recommend selecting clones which stain strongly at less than 1 µg/mL when tested by conventional IHC. However, each antibody is different, and performance of individual clones cannot be guaranteed.
- Fluor-labeled rabbit anti-Tag detector antibody sufficient to stain 15–20 slides is included. Additional fluor-labeled anti-Tag detector antibody is available.

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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	Fluor-labeled rabbit anti-Tag detector antibody, 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 2 minutes @ 1500 g. **Discard** flow-through.
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 collection tube, and centrifuge for 2 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 a collection tube, then place in microcentrifuge and spin for 2 minutes @ 1500 g.
5. Add linker-modified antibody solution from Step 4 to **GREEN**-capped column, replace **GREEN** cap loosely on column, insert in a collection tube, and centrifuge for 2 minutes @ 1500 g.
6. Add flow-through from **GREEN**-capped column collection tube from Step 5 to UltraTag 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 reagent tube from Step 6, 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 a collection tube, then place in microcentrifuge and spin for 2 minutes @ 1500 g. **Discard** flow through.
9. Transfer the UltraTag-antibody conjugation mixture from Step 7 to the **RED**-capped column and centrifuge for 2 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.

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Staining

- Recommended concentration for staining with UltraTag-labeled primary antibodies is 5–10 µg/mL, however, optimization may be necessary, and performing a titration may suggest a different optimal concentration.
- Recommended concentration for staining with fluor-labeled anti-Tag detector antibody is 5 µg/mL.

The standard protocol for antigen retrieval and staining is comprised of the following steps:

1. **Antigen Retrieval**: Use your preferred antigen retrieval method for primary antibody/anti-species secondary antibody staining. See suggested protocols below

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

2. **Staining**: One-hour incubation with UltraTag-labeled primary antibody cocktail followed by one-hour incubation with fluor-labeled anti-Tag-detector antibody cocktail.

Download detailed staining protocol from cellidx.com/documents/protocols/UltraTag-Multiplex-Staining-Protocol-Fluorescent.pdf.

Detailed protocols for manual antigen retrieval and fluorescent multiplex IHC staining can be downloaded from cellidx.com/technical/protocols.

Imaging

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

Anti-Tag Antibody	Fluor	Absorbance (nm)	Emission (nm)	Extinction Coefficient ($M^{-1}cm^{-1}$)	Recommended Exposure Time (ms)
UT015	CL-490	491	515	73,000	100-300
UT016	CL-550	550	575	150,000	100-300
UT019	CL-595	594	615	92,000	100-300
UT021	CL-650	655	676	250,000	200-400
UT019	CL-750	759	780	240,000	300-600

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

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Safety Information

- **For Research Use Only**. Not for diagnostic or therapeutic use.
- WARNING – CHEMICAL HAZARD. Some chemicals used can be potentially hazardous, and can cause injury or illness.
- Read and understand the Material Safety Data Sheets (MSDS) available at www.cellidx.com before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with and inhalation of chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.*, safety glasses, gloves, or clothing). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's clean-up procedures as recommended in the MSDS.
- Comply with all local, state/provincial, and/or national laws and regulations related to chemical storage, handling, and disposal.

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