UltraTag™ Multiplex Labeling Kit Protocol — Chromogenic

The UltraTag Multiplex Labeling Kits efficiently conjugate 50 µg of antibody in 50 µL with one of three available Tags in two hours, with highly efficient tagging, and >80% recovery. Antibodies labeled with different UltraTag kits (UTCK-HRP1, UTCK-HRP2, UTCK-AP1) can be combined to create three-plex chromogenic multiplex IHC panels using included enzyme-labeled anti-Tag antibodies.

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.

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

This protocol can be downloaded from cellidx.com/documents/protocols/UltraTag-Multiplex-Labeling-Kit-Protocol-Chromogenic.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.
- Enzyme-labeled rabbit anti-Tag antibody sufficient to stain 15–20 slides is included. Additional enzyme-labeled anti-Tag antibody is available. Components needed, but not supplied, include chromogens and HRP-arrest solution.
- Optimization recommendations: For chromogenic multiplex staining, we recommend planning out the desired chromogen color for each antibody and tagging accordingly. For example, if you plan on using a Red AP chromogen for a specific antibody, we recommend tagging that antibody with UT015 since this kit comes with an anti-UT015 AP antibody.

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	Enzyme-labeled rabbit anti-Tag detector antibody, 15 µg @ 1.0 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 **3** 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.

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 enzyme-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**: Download detailed staining protocol from cellidx.com/documents/protocols/UltraTag-Multiplex-Staining-Protocol-Chromogenic.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 Cell Palette chromogen catalog numbers and mounting media compatibilities of the chromogens which can be used with the enzyme-labeled anti-Tag antibodies included with the respective UltraTag kits. We recommend using CPAR-050 Cell Palette AP Red Chromogen, CPHB-050 Cell Palette HRP Blue Chromogen, and CPHY-050 Cell Palette HRP Yellow Chromogen. A combination kit (cat# CPK-015) of all three chromogens is available. For this chromogen combination, we have determined the optimal order to be Red, Blue, then Yellow. We recommend the use of a purple hematoxylin counterstain (cat# HP-050) followed by aqueous mounting medium or Cell IDx ChromoSealant (cat# CS-015) to seal stain followed by resin-based mounting medium.

Anti-Tag Antibody	Enzyme	Chromogen Recommendation	Catalog Number	Recommended Mounting Media
UT015	Alk Phos	Cell Palette AP Red	CPAR-050	Xylene-Based or Aqueous
UT016 or **UT021**	HRP	Cell Palette DAB	CPHD-050	Xylene-Based or Aqueous
UT016 or **UT021**	HRP	Cell Palette Blue	CPHB-050	Aqueous
UT016 or **UT021**	HRP	Cell Palette Yellow	CPHY-050	Xylene-Based or Aqueous

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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.