

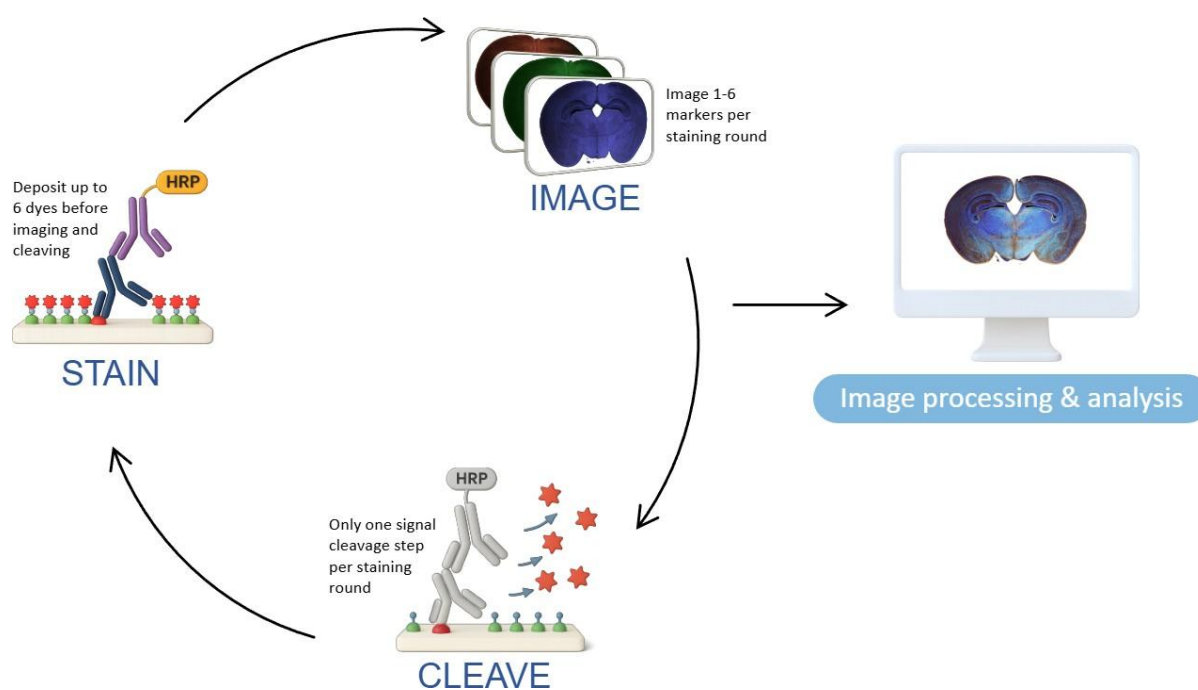
Ultra-Plex CFP™ Assay Quick Guide

BioCanvas™ Ultra Plex CFP™ Kit (Gen 2)

RK50080 (25T/5T)

For Research Use Only.

Not for use in diagnostic procedures.



Introduction

Reagent	Cat. No.	Volume/Quality		Qty. (tubes)	Storage
		25T	5T		
CFP 440 fluorophore	RM70080	50 µL	10 µL	1	-20 °C
CFP 490 fluorophore	RM70081	50 µL	10 µL	1	-20 °C
CFP 555 fluorophore	RM70082	50 µL	10 µL	1	-20 °C
CFP 645 fluorophore	RM70083	50 µL	10 µL	1	-20 °C
DAPI	RM02978	10 mL	5 mL	1	-20 °C
CFP Reaction Buffer	RM70084	30 mL	4 mL	1	2-8 °C
Endogenous Peroxidase Blocking Buffer	RM70001	10 mL	5 mL	1	2-8 °C
Antibody Stripping Buffer	RM02984	30 mL	6 mL	1	2-8 °C
Universal polyHRP Secondary Antibody	AS100	20 mL	4mL	1	2-8 °C
CFP Cleavage A	RM70086	2 mL	800 µL	1	2-8 °C
Cleavage Diluent	RM70087	500 µL	200 µL	1	2-8 °C
CFP Cleavage B	RM70085	40 mg×6	40 mg×2	25T:6	Room temperature
				5T:2	

The **Ultre-Plex Cleavable Fluorescent Probe (CFP™)** is designed for multiplexed immunofluorescence (mIF) assays. The proprietary multi-color fluorophores are covalently deposited, then efficiently removed by a mild cleavage process, enabling reiterative staining of up to 30+ protein and RNA targets.

This protocol is intended for use with the 3-color CFP Multiplex Protein Detection Complete Kit (Cat. No. K3C-1001). It has been validated for both manual and automated workflow on Leica BOND Rx and Biocare ONCORE™ PRO X staining systems.

Kit Content and Storage

The Complete Kit listed below includes 4 color CFP fluorophores and the reagents for 100 staining cycles and 50 cleavage process. A single stain is defined as 150 µL of staining volume on frozen or formalin-fixed paraffin-embedded (FFPE) tissues. A single cleavage is defined as 300 µL of cleavage reagent.

Store CFP fluorophores (Cat. No. RM70080、RM70081、RM70082、RM70083) at -20 °C and use within 6 months. Thaw an aliquot of the CFP stock solution before the experiment. Store the CFP dilution at 2-8 °C and use within 1 weeks.

A total of 12 CFP fluorophores are available for selection (see Appendix for details).

User Supplied Reagents

Description	Recommendation
Tris-buffered saline, 20x, pH 7.4	Fischer Scientific (Cat. No. AAJ60877K3)
Tween-20	Sigma Aldrich (Cat. No. NC1224369)
Phosphate-buffered saline, 1x	Thermo Scientific Chemicals (Cat. No. AAJ61196AP)
DI water (DNase/RNase free)	Thermo Scientific (Cat. No. FERA57775)
Antibody Blocking Buffer:	
Serum derived from the same host species as the secondary antibody should be preferentially selected for blocking.	

Before the Experiment

Prepare Wash Solution

Prepare 1L wash solution (1xTBS, 0.1% Tween(v/v)) according to the following recipe. Store at 2-8 °C and use within 2 months. Alternatively, BOND wash solution or ONCORE Pro wash buffer can be used as the wash solution.

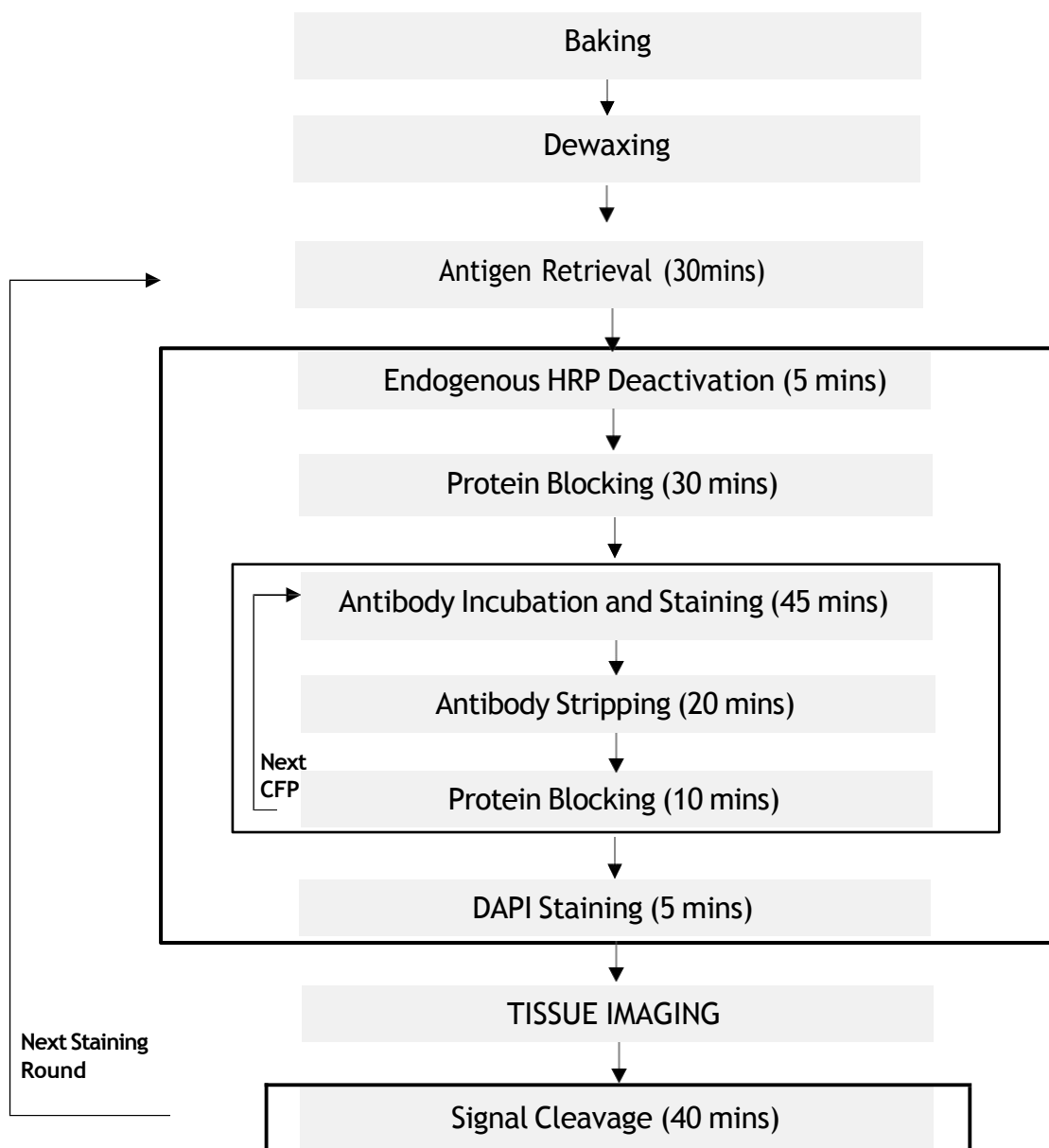
Description	Volume
Tris-buffered saline, 20x, pH7.4	50 mL
Tween-20	1 mL
DI water	950 mL

Ultre-Plex CFP Assay Workflow

The workflow is compatible with both FFPE tissue and frozen tissue samples. For FFPE tissues, sample preparation includes tissue baking, dewaxing and antigen retrieval. The Ultre-Plex CFP assay workflow begins with endogenous peroxidase deactivation and protein blocking. The target protein is detected by the user-selected or user-supplied primary antibody and HRP conjugated secondary antibody. Subsequently, the CFP fluorophore is introduced to covalently deposit in the vicinity of the target protein. Between staining cycles, antibodies from the previous staining cycle are removed with a stripping process. After imaging, all fluorescent signals are removed by a cleavage process. Then the tissue sample is ready for the next round of staining.

The Ultre-Plex CFP assay is compatible with protease-free RNA-ISH kits for RNA/protein co-detection, such as the Molecular Instruments' HCR™ Pro RNA-ISH assay. For more information, contact: info@abclonal.com.

Assay Workflow



*Note:

- For frozen samples, workflow begins with Endogenous HRP Deactivation.
- For each staining round: option to skip “Antibody Stripping” and “Protein Blocking” before the DAPI staining.

Assay Steps

1. Sample Preparation

- Perform the tissue Baking at 60°C.
**Note: A minimum of 30 minutes tissue baking is recommended to minimize tissue detachment during staining.*
- Perform tissue dewaxing either manually using a selected dewaxing solution (e.g., xylene or HistoClear) or using an automated dewaxing protocol on a staining platform (e.g., Leica BOND or Biocare Oncore™ Pro).

2. Antigen Retrieval

- Perform the Antigen Retrieval with Antigen Retrieval Solution* at 100°C for 30mins.
- Gently wash the sample 3 times with the wash solution (2 mins incubation is recommended in the last wash step).

**Note: Group antibodies based on their antigen retrieval conditions and stain antibodies requiring pH 6 antigen retrieval first.*

3. Endogenous HRP Deactivation

- Apply the Endogenous Peroxidase Blocking Buffer (Cat. No. RM70001) to the sample and incubate for 5 mins.
- Gently wash the sample with DI water 3 times.

4. Protein Blocking

- Apply the Antibody Blocking Buffer (User Supplied Reagents) to the sample and incubate for 30 mins.

5. Antibody Incubation and Staining

- Preparation of Primary Antibodies
 - Dilute Primary Antibody with Antibody Blocking Buffer (User Supplied Reagents) according to the vendor recommended dilution factor.
 - Optimize dilution ratios as needed for specific samples or markers.
- Preparation of CFP Fluorophores
 - Prepare CFP diluent by mixing Endogenous Peroxidase Blocking Buffer (Cat. No. RM70001) and CFP Reaction Buffer (Cat. No. RM70084) at 1:1000 ratio (1 µL of 3% H₂O₂ into 1 mL CFP Reaction Buffer). Use the CFP diluent within 4 hours.
 - Dilute the CFP fluorophore with CFP diluent to the working concentration.
 - Recommended dilution ratios: 1:100-1:500 for CFP 440, CFP 490, CFP555, CFP645.
 - For a 1:200 dilution, use 1 mL CFP dilution buffer + 5 µL CFP fluorescein (500x) .

- Dilution ratio may require optimization for specific sample and marker. (Recommended range 1:100 to 1:1000).

- Primary Antibody Incubation
 - Apply the diluted Primary Antibody to the sample and incubate for 20 mins.
 - Gently wash the sample 3 times with the wash solution (2 mins incubation is recommended in the last wash step).
 - HRP Secondary Antibody Incubation
 - Apply the Universal polyHRP Secondary Antibody* (Cat. No. AS100) to the sample and incubate for 15 mins.
 - Gently wash the sample 3 times with the wash solution (2 mins incubation is recommended in the last wash step).
- *Note: Species-specific secondary antibodies may be substituted as needed.*
- CFP Fluorophore Staining
 - Apply the diluted CFP fluorophore to the sample and incubate for 10 mins.
 - Gently wash the sample 3 times with the wash solution (2 mins incubation is recommended in the last wash step).

6. Antibody Stripping*

- Gently wash the sample with the Antibody Stripping Buffer (Cat. No. RM02984P).
- Apply the Antibody Stripping Buffer to the sample and incubate for 20 mins at 50 °C
- Gently wash the sample 3 times with the wash solution (2 mins incubation is recommended in the last wash step).

**Note: Heat-induced antigen retrieval (HIER) has been validated as an alternative stripping method for ≤8-plex applications.*

7. Protein Blocking

- Apply Antibody Blocking Buffer (User Supplied Reagents) to the sample and incubate for 10 mins.
- Gently wash the sample 3 times with the wash solution (2 mins incubation is recommended in the last wash step).

**Note: Each staining round, optional to skip steps 6 and 7 in the last staining cycle.*

8. Repeat Staining Cycles

- Repeat Steps 5 - 7 with each additional CFP fluorophore for the next staining cycle.

9. DAPI Staining

- Prepare DAPI working solution by diluting DAPI (Cat. No. RM02978) 1:1000 in 1x PBS.
- Apply DAPI working solution to the sample and incubate for 5 mins.
- Gently wash the sample 3 times with the wash solution.

10. Tissue Imaging

- After finishing all 4 CFP and DAPI staining, apply mounting medium and coverslip to the slides before imaging.
- Proceed with fluorescence imaging.

11. Signal Cleavage*

Signal cleavage includes two sequential steps. First, incubate the sample with Cleavage A solution at 50 °C for 30 mins. Second, incubate slides in the pre-aliquot Cleavage B reagent container for 10 mins at ambient temperature.

1. Cleavage with RM70086

- Preparation of Cleavage Solution A:
Mix Cleavage A (Cat. No. RM70086) with Cleavage Diluent (Cat. No. RM70087) and DI water at a ratio of 4:1:15. Store the cleavage solution at 2-8 °C and use within 2 days (e.g., for 1 mL cleavage solution: mix 200 µL Cleavage A, 50 µL Cleavage Diluent and 750 µL DI water).
- Cleave with Cleavage Solution A:
 - Gently wash the sample with the Cleavage Solution A.
 - Apply Cleavage Solution A to the sample and incubate for 30 mins at 50 °C.
 - Gently wash the sample 3 times with the wash solution. A 2-min incubation is recommended in the last wash step.

2. Cleavage with RM70085

- Add 40 mL of 1x PBS to each slide stain container, which contains pre-aliquoted Cleavage B reagent. Cap the container and gently shake until the reagent is fully dissolved.
**Note: Prepare the cleavage solution fresh and use within 10 mins.*
- Remove the cap and incubate up to 5 slides per container with the freshly prepared cleavage solution for 10 mins at room temperature.
**Note: Do not recap the container during incubation.*

- Wash slides 3 times with the wash solution at room temperature.

**Note: Cleavage efficiency may vary depending on antibodies and tissue types. It is recommended to verify the signal cleavage efficiency when working with new antibodies or tissue types. Repeat the cleavage steps if necessary to enhance the signal removal efficiency.*

12. Iterative Staining and Imaging

- Repeat Steps 2 - 10 for the next staining and imaging round.

Automated scripts for this kit on Leica BOND Rx and Biocare ONCORE™ PRO X are available upon request. Contact: info@abclonal.com

APPENDIX:

Available CFP fluorophores

Fluorophores	Equivalent Fluorophores	Ex/Em(nm)
CFP 440	Alexa 440	435/480
CFP 450	Opal 480	450/502
CFP 490	Opal 520, Alexa 488, FITC	490/515
CFP 515	Opal 540	510/527
CFP 545	Alexa 546	540/557
CFP 555	Opal 570, Alexa 555, Cy3	557/570
CFP 595	Opal 620, Alexa 594, Texas Red	587/603
CFP 635	Opal 650, Alexa 633, Cy5	640/655
CFP 645	Alexa 647, Cy5	655/670
CFP 670	Opal 690	670/682
CFP 680	Opal 690, Alexa 680, Cy5.5	685/701
CFP 750	Opal 780, Cy7, Alexa 750	757/780



For Research Use Only.

Not for use in diagnostic procedures.

All rights reserved.

CONTACT INFORMATION

Phone: +1 888-754-5670

E-mail: info@abclonal.com

Address: 500W Cummings Park, Ste. 6500 Woburn, MA 01801

Contact Us: [LinkedIn](#)