Kit Start

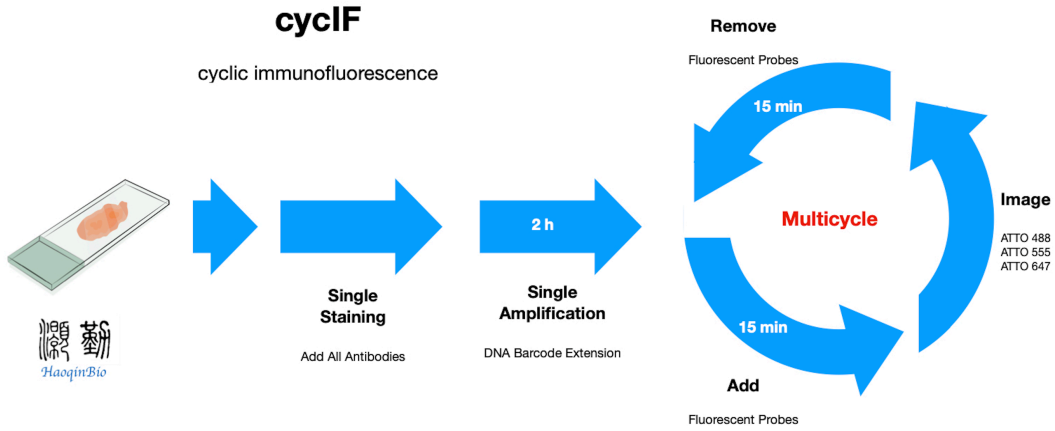
— 6 biomarkers

Expertise needed to implement:

The expertise needed is similar to that needed for IHC and immunofluorescence analyses. Specifically, knowledge of specimen selection, tissue sectioning, antibody staining and microscopy are required.

1. Introduction:

The start kits are intended for fluorescent multiplex immunohistochemistry. There are 6 DNA Barcodes included for 6 different antibody labeling. However, additional Paired Barcode and Probe kits can be ordered separately for more target antigens.



2. Solution and Reagents (Included)

Kit Start (6 biomarkers) = Kit A x 6 + Kit B x 1

1	Kit A-**51-488**
2	Kit A-**52-555**
3	Kit A-**53-647**
4	Kit A-**54-488**
5	Kit A-**55-555**
6	Kit A-**56-647**
7	Kit B

Noted : Sufficient for **10μg each antibody** labeling and the amplification of **10 slides (150 μl / slide)**

3. Required Reagents (Not Included)

1	TBST	Tris Buffered Saline with Tween 20	Cell Signaling Technology # 9997
2	DMSO	Dimethyl sulfoxide	Thermo Fisher Scientific, cat. no. 12345
3	BS3	Bis(sulfosuccinimidyl)suberate /an amine-to-amine crosslinker	Thermo Fisher Scientific, cat. no. 21580

4. Microscopy

When imaging, there are 3 fluorescent channels in addition to DAPI that need to be acquired. Confirm your microscope can detect the fluorophores provided in this kit.

Fluorophore Channel	Excitation (nm)	Emission (nm)	Common Filter Set
488	488	520	FITC
555	560	575	Cy®3
647	650	668	Cy®5

5. Solution Preparation

1. Amplification Solution A

Amplification Enzyme A (20 x) / μL	Amplification Buffer A (10 x) / μL	Nuclease-free Water / μL	→	Amplification Solution A μL
7.5	15	127.5		150

2. Amplification Solution B

Amplification Enzyme B (30 x) / μL	Amplification Buffer B (10 x) / μL	Nuclease-free Water / μL	→	Amplification Solution B μL
5	15	130		150

3. Fluorescent Probes Mixed Solution (3 Probes in Each Cycle)

TBST / μL	DMSO (20%) / μL	Fluorescence Probe (20 x) / μL	→	Fluorescent Probes Mixed Solution μL
97.5	30	7.5 x 3		150

4. Wash Solution

TBST / μL	DMSO (30%) / μL		→	Wash Solution μL
700	300			1000

5. Removal Solution

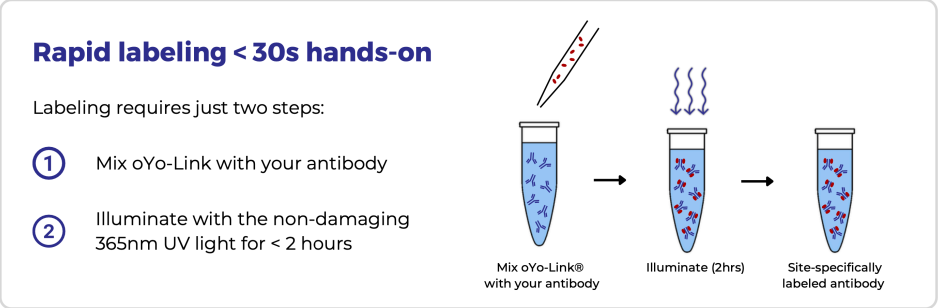
TBST / μL	DMSO (70%) / μL		→	Removal Solution μL
300	700			1000

6. Protocol for use

1. FFPE Slide Preparation

2. Label Antibody with Barcode DNA

1. oYo-Link® Antibody Compatibility. (https://alphathera.com/antibody-and-buffer-compatibility/)
2. Rapid labeling. (https://alphathera.com/resources/#usermanuals-and-protocols)



3. Staining

Incubate slides in 150μL Barcoded Antibodies Mixed Solution overnight at 4°C

4. Post-staining antibody fixation

Fixation with irreversible BS3 cross linker is necessary to maintain antibodies bound to tissue for many cycles of hybridization and stripping

5. Amplification

1. Incubate slides in 150μL **Amplification Solution A** for 60 min at 43°C.
2. Wash the slides two times in TBST, each for 30 sec, at RT.
3. Incubate slides in 150μL **Amplification Solution B** for 100 min at 37°C.
4. Wash the slides two times in TBST, each for 30 sec, at RT.

6. Adding Fluorescent Probes

1. Incubate slides in 150μL **Fluorescent Probes Mixed Solution** for 15 min at 37°C.
2. Wash the slides two times in TBST, each for 30 sec, at 37°C.
3. Wash the slides in **Wash Solution** for 1 min at 37°C.
4. Wash the slides two times in TBST, each for 30 sec, at 37°C.

7. Image

1. Counterstain with DAPI solution.
2. Mount slides with Anti-fade Reagent.
3. Image slides.

8. **Removal of Fluorescent Probe (**option**)**

    1. Incubate slides in **Removal Solution** for 1 min at 37°C.
    2. Wash the slides in TBST for 30 sec at 37°C.
    3. Incubate slides in **Removal Solution** for 1 min at 37°C.
    4. Wash the slides in TBST for 30 sec at 37°C.

Cycle:

9. Adding New Fluorescent Probes
10. Image
11. Removal of Fluorescent Probes