Multiplex IHC Detection Kit (Septuple)

User Manual

Catalog # CRG1090

(Version 1.3A)

Multiplex IHC Detection Kit (Septuple) is seven colors multiplex IHC test kit using tyramide signal amplification (TSA).

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Tyramide signal amplification (TSA), also called catalyzed reporter deposition (CARD), is a highly sensitive enzymatic method which can enable the detection of low-abundance targets in histochemical analysis. TSA utilizes the catalytic activity of HRP for the covalent deposition of labeled tyramide on and near target proteins or nucleic acid sequences in situ. In the presence of low hydrogen peroxide (H2O2), HRP is able to convert labeled tyramide substrates into highly-reactive, short-lived tyramide radicals that rapidly bind to tyrosine residues on and proximal to the enzyme site. These labels can be detected by standard chromogenic or fluorescent techniques. Multiple rounds of tyramide signal amplification can be performed for multicolor detection.

II. KIT COMPONENTS

Component	20 Assays	100 Assays	Storage	Cap
DAPI (200X)	10 µl x 1	50 µl x 1	4 °C	Bule
TSA-480 Dye (200X)	10 µl x 1	50 µl x 1	4 °C	Purple
TSA-520 Dye (200X)	10 µl x 1	50 µl x 1	4 °C	Green
TSA-570 Dye (200X)	10 µl x 1	50 µl x 1	4 °C	Yellow
TSA-620 Dye (200X)	10 µl x 1	50 µl x 1	4 °C	Orange
TSA-690 Dye (200X)	10 µl x 1	50 µl x 1	4 °C	Red
TSA-780 Dye (200X)	10 µl x 1	50 µl x 1	4 °C	White
Goat Anti-Mouse/Rabbit HRP Polymer	12 ml x 1	30 ml x 2	4 °C	Bule
Tyramide Amplification Buffer	12 ml x 1	30 ml x 2	4 °C	Brown
Antifade Mounting Medium	100 µl x 1	500 µl x 1	4 °C	Clear
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III. STORAGE AND STABILITY

All kit components are stable at 2-8 °C for 1 year.

IV. MATERIALS REQUIRED BUT NOT PROVIDED

1. Xylene
2. Ethanol
3. Hydrogen Peroxide
4. Pipettor
5. Timer
6. Microwave
7. IHC pen
8. PBST
9. BSA
10. Fluorescence microscope

V. WORKING SOLUTION PREPARATION

All reagents need to be centrifuged before use.

1. DAPI Working Solution

Add 1 µl DAPI (200X) into 200 µl PBS before use, mix.

2. TSA-480 Dye Working Solution

Add 1 µl TSA-480 Dye (200X) into 200 µl Tyramide Amplification Buffer before use, mix.

3. TSA-520 Dye Working Solution

Add 1 µl TSA-520 Dye (200X) into 200 µl Tyramide Amplification Buffer before use, mix.

4. TSA-570 Dye Working Solution

Add 1 µl TSA-570 Dye (200X) into 200 µl Tyramide Amplification Buffer before use, mix.

5. TSA-620 Dye Working Solution

Add 1 µl TSA-620 Dye (200X) into 200 µl Tyramide Amplification Buffer before use, mix.

6. TSA-690 Dye Working Solution

Add 1 µl TSA-690 Dye (200X) into 200 µl Tyramide Amplification Buffer before use, mix.

7. TSA-780 Dye Working Solution

Add 1 µl TSA-780 Dye (200X) into 200 µl Tyramide Amplification Buffer before use, mix.

VI. ASSAY PROCEDURE

1. De-paraffinizing (de-waxing) and rehydrating

1.1 Heat the slides in tissue-drying oven for 45 minutes at 60°C. Place the slides in a rack, and perform the following washes:

Xylene I: 15 mins

Xylene II: 15 mins

100% Ethanol: 5 mins

95% Ethanol: 5 mins

85% Ethanol: 5 mins

70% Ethanol: 5 mins

1.2 The slides are placed in a lab draught cupboard to rinse off ethanol.

1.3 Finally, wash the slides in the pure water.

2. Antigen or epitope retrieval

2.1 Add the appropriate antigen retrieval buffer (EDTA pH 9.0 or sodium citrate pH 6.0) to the microwaveable vessel.

2.2 Place the slides in the microwaveable vessel. Then place the vessel inside the microwave (1200W).

2.3 Boil for 8 mins in microwave (1200 W) under medium heat, then stop heating for 8 mins, followed by low and medium heat for 7 mins. Other heat-induced epitope retrieval methods can also be used, e.g., heated at 120 °C 1-2 min, 100 °C 20mins or 95 °C in a water bath.

Be sure to monitor for evaporation and watch out for boiling over during the procedure. Do not let the slides dry out.

2.4 After cooling down in room temperature, place the slides in PBS (pH 7.4) to wash 3 X 5 mins on a decolorizing shaker.

Notes: To get best results, antigen retrieval buffer and protocol should be determined according to the tissue types and antigen types.

3. Blocking endogenous peroxidase

3.1 Add enough 3% hydrogen peroxide (H2O2) to cover the slides.

3.2 Incubate for 15 mins in the dark at room temperature.

3.3 Place the slides in PBS (pH 7.4) to wash 3 X 5 mins on a decolorizing shaker.

4. Blocking

4.1 Drain slides and then use an IHC pen to draw a circle around each sample on your slide (to hold antibody solution within the target area).

4.2 Add 3% BSA-PBST solution (or other blocking buffer) inside the circle to cover the tissues, incubate 30 mins at room temperature.

5. Primary antibody incubation

5.1 Remove blocking buffer and add primary antibody diluted by recommended antibody diluent overnight at 4°C or 37°C for 1-2h.

6. HRP Polymer incubation

6.1 Place the slides in PBS (pH 7.4) and wash 3 X 5 mins on a decolorizing shaker.

6.2 Incubate slides with HRP Polymer (100 µl for each slice) in the dark at room temperature for 60 mins.

6.3 Wash 3 X 5 mins with PBS buffer.

7. Tyramide labeling

7.1 Apply the TSA-480 Dye Working Solution to each sample (100 µl for each slice) and incubate for 10-15 mins at room temperature.

7.2 Wash 3 X 5 mins with PBS buffer.

8. Denoise

Repeat steps 2. At the end of the single stain, may add antifade mounting medium to view the slides or go on labeling another fluorescent dye.

9. Repeat

Repeat steps 3-7 for another fluorescent dye (TSA-520 Dye Working Solution, TSA-570 Dye Working Solution, TSA-620 Dye Working Solution, TSA-690 Dye Working Solution, TSA-780 Dye Working Solution) to each sample.

10. DAPI counterstaining

10.1 Place the slides in PBS (pH 7.4) and wash 3 X 5 mins on a decolorizing shaker.

10.2 Apply the DAPI solution (100 µl for each slice) to each sample and incubate in the dark at room temperature for 10 mins.

11. Mounting the slides

11.1 Place the slides in PBS (pH 7.4) and wash 3 X 5 mins on a decolorizing shaker.

11.2 Add antifade mounting medium (3-5 µl for each slice) to cover the section.

12. View the slides

View the sample using a fluorescence microscope with appropriate filters.

Note: To get the best results from multiplex staining, the experimental condition must be optimized.

Fluorophore Excitation and Emission Maxima

Fluorophore	Excitation	Emission
DAPI	364 nm	454 nm
TSA-480 Dye	429 nm	475 nm
TSA-520 Dye	490 nm	515 nm
TSA-570 Dye	555 nm	565 nm
TSA-620 Dye	590 nm	617 nm
TSA-690 Dye	680 nm	701 nm
TSA-780 Dye	764 nm	788 nm

VII. NOTICE

1. Please centrifuge the reagent to the bottom of the tube before use.
2. Negative control should be set up during the experiment (without antibodies or TSA dye).
3. Higher concentrations TSA dye may cause the background or the signal too strong. We recommend the dilution from 1:100 to 1:1000.
4. TSA dyes can be used to label different targets on the same sample. The antibodies need to be stripped after each tyramamide reaction.
5. When multi-color labeling, TSA dyes need to be selected according to different antigen density. Low density antigens need to label stronger dyes; high density antigens need to label weaker dyes. The order of label will affect the result, which needs to be explored by yourself.
6. If labeling cell samples or frozen sections, need to proceed a pre-experiment and determine whether the reagent is available or not.

VIII. TROUBLESHOOTING

Low Signal

- Titer primary and/or TSA dyes to determine optimum concentration for TSA detection.
- Increase primary antibody and/or TSA dye Working Solution incubation time.
- Change staining order of multiplex and move this specific target detection towards the end of staining procedure.

Excess Signal

- Decrease concentration of primary antibody.
- Decrease concentration of TSA dyes.
- Decrease TSA dye Working Solution incubation time.

High Background

- Add extra step to block endogenous peroxidases.
- Titer primary and/or TSA dyes to determine optimum concentration for TSA detection.
- Make fresh buffers.
- Evaluate laboratory water source for contamination.
- Increase number and/or length of washes.

IX. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com