Research Use Only. Not for use in diagnostic procedures.

Opal™ Polaris 780 Reagent Pack (FP1501001KT)

Materials Provided

- Opal TSA-DIG Reagent, 1 vial
- Opal Polaris 780 Reagent, 1 vial
- Dimethyl sulfoxide, DMSO, 1 x 100 uL

Please note that Opal TSA-DIG Reagent and Opal Polaris 780 Reagent are provided dry. DMSO has been included to reconstitute Opal TSA-DIG. Reconstitute Opal Polaris 780 in ddH_2O. Please follow the protocol provided below.

Product Information

Protocol for Opal TSA-DIG reconstitution:

1. Dissolve the Opal TSA-DIG Reagent in 75 uL of DMSO.

2. Carefully dispense DMSO along the sides of the vial and mix several times to dissolve any Opal TSA-DIG Reagent that might coat the sides of the vial. Wait at least 20 minutes before use to allow the entire solution to dissolve.
   Minimize bubbles while mixing.

Protocol for Opal Polaris 780 reconstitution:

1. Dissolve the Opal Polaris 780 Reagent in 300 uL of ddH_2O.

2. Opal Polaris 780 is a lyophilized conjugated antibody. Carefully dispense ddH_2O along the sides of the vial and mix several times to dissolve any Opal Polaris 780 Reagent that might coat the sides of the vial.
   Minimize bubbles while mixing.

Storage Store dry reagent in the dark at -20 ºC. Upon reconstituting in DMSO or ddH_2O, store in the dark at 4ºC.

Stability See label on outside of box for expiration date.

Safety Note DMSO is classified as hazardous and combustible. Opal Polaris 780 contains sodium azide as preservative. It is strongly recommended to wear disposable gloves and safety glasses while working with the items in this kit. Thorough washing of hands after handling is also recommended.

Quality Control We certify that QC results of these reagents meet our quality release criteria.

Opal TSA-DIG Working Solution
Before each procedure, dilute Opal TSA-DIG in 1X Plus Amplification Diluent for manual staining or 1X Plus Automation Amplification Diluent for automation to make Opal TSA-DIG Working Solution. The recommended dilution ratio is 1:100.

Opal Polaris 780 Working Solution
Opal Polaris 780 is a conjugated antibody. Before each procedure, dilute Opal Polaris 780 in Blocking/Ab Diluent to make Opal Polaris 780 Working Solution. The recommended dilution ratio is 1:25.

Generally, 100-300 µL of Opal Working Solution is required per slide. Discard any unused portion of Opal Working Solution.

Procedure

Step 1: Slide Preparation
Prepare tissues or cells for detection with Opal using standard fixation and embedding techniques. We recommend running an isotype control slide with all steps replacing the primary antibody with corresponding isotype control for each experiment. For each slide, baked in the oven at 65^{o}C for 1 hour; dewax with xylene (3 x 10 min) and rehydrate through a graded series of aqueous ethanol solutions: (100% 1 x 10 min; 95% 1 x 10 min; and rinse in 70%). After rehydration, briefly rinse slides in distilled water and fix in 10% neutral buffered formalin (NBF) for 20 min. Longer times of fixation in NBF may be needed for certain tissues such as skin.

Rinse slides in distilled water and then in the appropriate AR buffer.

Step 2: Microwave treatment
Place slides in an Opal Slide Processing Jar and fill it completely with the appropriate AR buffer. Loosely cover the jar with lid, place it in microwave for 45 sec at 100% power; may require optimization as described. Microwave for an additional 15 min at 20% power. Allow slides to cool down at room temperature before proceeding (15 – 30 min). Importantly, do not let slides dry out. Rinse slides in distilled water followed by TBST (Tris-buffered saline, 0.1% Tween 20).

Step 3: Blocking
Use a hydrophobic barrier pen to completely surround the tissue section on the slide. Cover tissue sections with blocking buffer and incubate slides in a humidified chamber for 10 min at room temperature.

- *Note: This protocol was developed with PerkinElmer Antibody Diluent/Block for blocking. Other options should be independently validated.*

Step 4: Primary Antibody Incubation
Drain off the blocking buffer and apply Primary Antibody Working Solution. Incubate according to the manufacturer's instructions regarding incubation time and temperature requirements or conditions optimized within your lab. Use enough volume to completely cover the tissue section (generally 100-300 µL per slide).

Rinse slides in TBST. Wash the slides 3 x 2 min in TBST at room temperature preferably with agitation.

Step 5: Introduction of Secondary-HRP

Incubate slides in Polymer HRP Ms + Rb for 10 min at room temperature. Use adequate reagent volume to cover the tissue section, generally 100-300 µL per slide.

- *Note: Opal Polymer HRP Ms + Rb is recommended for experiments with human tissue and mouse or rabbit primary antibodies. Other options should be independently validated.*

Rinse slides in TBST. Wash the slides 3 x 2 min in TBST at room temperature preferably with agitation.

Step 6: TSA Binding to Target
Drain off excess wash buffer and pipette 100-300 µL of Opal TSA-DIG Working Solution onto each slide. Incubate the slides at room temperature for 10 mins.

Rinse slides in TBST. Wash the slides 3 x 2 min in TBST at room temperature preferably with agitation.

Rinse slides in the appropriate AR buffer.

Step 7: Microwave treatment
Place slides in an Opal Slide Processing Jar and fill it completely with the appropriate AR buffer. Loosely cover the jar with lid, place it in microwave for 45 sec at 100% power; may require optimization as described. Microwave for an additional 15 mins at 20% power. Allow slides to cool down at room temperature before proceeding (15 – 30 min). Importantly, do not let slides dry out. Rinse slides in distilled water followed by TBST.

Step 8: Fluorophore Signal Generation
Drain off excess wash buffer and pipette 100-300 µL of Opal Polaris 780 Working Solution onto each slide. Incubate the slides at room temperature for 1 hr.

Rinse slides in TBST. Wash the slides 3 x 2 min in TBST at room temperature preferably with agitation.

Rinse slides in the appropriate AR buffer.

Step 8: Counterstain and Mount
Apply DAPI Working Solution for 5 min at room temperature in a humidity chamber. Wash the slides for 2 min in TBST buffer and then for 2 min in water. Coverslip slides with mounting medium (i.e. ProLong® Diamond Antifade Mountant (Thermofisher)). *(Note: do not counterstain monoplex slides to be used for spectral library development.)*

Cautionary Note

When using in an Opal IHC Multiplex Fluorescent Procedure, Opal Polaris 780 **MUST GO LAST.** **No removal steps should be performed after the incubation of Opal Polaris 780 working solution.**

When running Opal Polaris 780 on a Leica BOND RX, please ensure there are three wash steps, including a one-minute incubation, between the Opal TSA DIG denaturation step and the application of Opal Polaris 780.

Troubleshooting

Technical Support Resources

- **Email:** **global.techsupport@perkinelmer.com**
- **Telephone**
  - **USA toll-free** **800-762-4000**
  - **EU toll-free** **00800 33 29 0000**
  - **China toll-free** **800 820 5046**

- **Local contact numbers:** http://www.perkinelmer.com/corporate/locations