

## Opal Reagent Pack (480, 520, 540, 570, 620, 650, 690, 780)

CATALOG #s: FP1500001KT, FP1487001KT, FP1494001KT, FP1488001KT, FP1495001KT, FP1496001KT, FP1497001KT, FP1501001KT

### INTRODUCTION

Opal® fluorescent dyes provide sub-cellular sensitivity to detect any biomarker of interest using tyramide signal amplification (TSA). Opal dyes are extensively utilized in spatial biology applications to detect co-localization and low abundance biomarkers. Each Opal Reagent Pack includes 1 vial of Opal dye and 1 vial of DMSO for dye reconstitution.

**NOTE** Storage and Stability information can be accessed at [akoyabio.com](http://akoyabio.com). Refer to the individual Technical Data Sheet available for each Opal Reagent Pack.

### SUPPLIED MATERIALS

The following components are supplied per Opal Reagent Pack:

Catalog #	Description	Components		
		Component #	Description	Quantity
FP1500001KT	Opal 480 Reagent Pack	OP-001000 DMSO0100UL	Opal 480 Reagent DMSO	1 vial 1 x 100 µL
FP1487001KT	Opal 520 Reagent Pack	OP-001001 DMSO0100UL	Opal 520 Reagent DMSO	1 vial 1 x 100 µL
FP1494001KT	Opal 540 Reagent Pack	OP-001002 DMSO0100UL	Opal 540 Reagent DMSO	1 vial 1 x 100 µL
FP1488001KT	Opal 570 Reagent Pack	OP-001003 DMSO0100UL	Opal 570 Reagent DMSO	1 vial 1 x 100 µL
FP1495001KT	Opal 620 Reagent Pack	OP-001004 DMSO0100UL	Opal 620 Reagent DMSO	1 vial 1 x 100 µL
FP1496001KT	Opal 650 Reagent Pack	OP-001005 DMSO0100UL	Opal 650 Reagent DMSO	1 vial 1 x 100 µL
FP1497001KT	Opal 690 Reagent Pack	OP-001006 DMSO0100UL	Opal 690 Reagent DMSO	1 vial 1 x 100 µL
FP1501001KT	Opal 780 Reagent Pack	OP-001008 OP-001007 DMSO0100UL	Opal 780 Reagent Opal TSA-DIG DMSO	1 vial 1 vial 1 x 100 µL

### MATERIALS NOT PROVIDED

Double distilled water (ddH<sub>2</sub>O) is required for the reconstitution of Opal 780 and is not provided.

## Opal Reagent Pack (480, 520, 540, 570, 620, 650, 690, 780)

CATALOG #s: FP1500001KT, FP1487001KT, FP1494001KT, FP1488001KT, FP1495001KT, FP1496001KT, FP1497001KT, FP1501001KT

### REAGENT PREPARATION

**NOTE** *Opal dyes and DMSO are light-sensitive. Please take precautions to protect from light exposure.*

- Store dry Opal at -20°C.
- Store DMSO at room temperature (RT).

### Reconstitution

1. Allow dry Opal dye to equilibrate to RT (~ 10 minutes).
2. Spin down dry Opal dye for 10 seconds at RT.
3. Reconstitute Opal dye and Opal TSA-DIG (provided in Opal 780 Reagent Pack) according to the table below.

**NOTE** *Ensure DMSO is completely liquid before using it to reconstitute Opal dye.*

TABLE 1. Reconstitution of Opal Dyes

	Opal 480	Opal 520	Opal 570	Opal 620	Opal 690	Opal TSA-DIG	Opal 780
<b>Diluent</b>	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	*ddH <sub>2</sub> O
<b>Diluent Volume (µL)</b>	75	75	75	75	75	75	300
*Reconstitute Opal 780 in ddH <sub>2</sub> O.							

4. Dispense DMSO (or ddH<sub>2</sub>O for Opal 780) along the sides of the vial (begin towards the top) to dissolve any Opal reagent that might coat the sides of the tube.
5. Gently vortex Opal dye (**do not vortex >10 seconds**).
6. Centrifuge 10 seconds at RT.
7. Ensure dye has completely dissolved by allowing Opal stock solution to stand for 15 minutes at RT.
8. Gently vortex (**do not vortex >10 seconds**) then centrifuge for 10 seconds at RT.
9. Aliquot for long term storage if desired. Screw caps are preferred.

**NOTE** *Store reconstituted Opal dye solution at 4°C protected from light. Use Opals 480, 520, 540, 570, 620, 650, 690 and TSA-DIG within 90 days of reconstitution. Use Opal 780 within 30 days of reconstitution.*

## Opal Reagent Pack (480, 520, 540, 570, 620, 650, 690, 780)

CATALOG #s: FP1500001KT, FP1487001KT, FP1494001KT, FP1488001KT, FP1495001KT, FP1496001KT, FP1497001KT, FP1501001KT

### Working Solutions

**NOTE** Consult individual assay protocols for detailed slide preparation procedures. Generally, 100-300  $\mu$ L of Opal working solution is required per slide.

Prepare working solution fresh before each experiment. Dispose of any unused working solution.

10. Ensure reconstituted Opal dye solution is equilibrated to RT (~10 minutes if retrieved from 4°C storage).
11. Gently vortex (**do not vortex >10 seconds**) then centrifuge for 10 seconds at RT.
12. Dilute reconstituted Opal dye stock solution (except Opal 780) at the indicated ratios and with the appropriate amplification diluent:
  - **Automated staining:** dilute at 1:150 in 1X Plus Automation Amplification Diluent (Catalog # FP1609)
  - **Manual staining:** dilute at 1:100 in 1X Plus Manual Amplification Diluent (Catalog # FP1498)

Dilute reconstituted Opal 780 dye stock solution in 1X Antibody Diluent/Block (Catalog # ARD1001EA) at a 1:25 dilution.

13. Discard unused working solution after staining is complete.

## ASSAY-SPECIFIC PROTOCOLS

### PhenoCode Signature Panels

Individual protocols are available for each PhenoCode™ Signature panel at akoyabio.com.

### Opal Staining using Secondary Antibody

Consult the Opal Assay Development Guide for protocol at akoyabio.com.

For Research use only. This product is distributed and sold for research purposes only by the end-user in the research market, and, to that extent, by purchasing this product the end-user is granted a limited license to use this product for research use only. This product is not intended for diagnostic or therapeutic use and no license or right is granted for use of this product for diagnostic or therapeutic purposes. Purchase does not include or carry any right or license to use, develop or otherwise exploit this product commercially. Any commercial use, development or exploitation of this product without the express prior written authorization of Akoya Biosciences is strictly prohibited and may constitute infringement of the intellectual property rights of Akoya Biosciences under the aforementioned patents.

TSA is a trademark of Akoya Biosciences. Opal is a registered trademark of Akoya Biosciences. Phenolmager is a registered trademark of Akoya Biosciences. Other trademarks are property of their respective owner.

To learn more visit: [AKOYABIO.COM](https://www.akoyabio.com) or email us at [INFO@AKOYABIO.COM](mailto:INFO@AKOYABIO.COM)

For Research Use Only. Not for diagnostic procedures.

©2023 Akoya Biosciences, Inc. All rights reserved. All trademarks are the property of Akoya Biosciences unless otherwise specified.