

# RPE-IgG Conjugation Kit

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## Introduction

R-Phycoerythrin is widely used as a fluorescent label in immunochemistry assays such as ELISA and in more complex techniques such as flow cytometry. Preparing bright, stable and reproducible antibody-PE conjugates is one of the biggest challenges of developing bead-based immunoassays and high quality reagents for flow cytometry. The OriGene RPE-IgG conjugation kit utilizes a novel chemistry to generate bright and highly reproducible RPE-IgG conjugates with a simple procedure. The resulting conjugates have been shown to be extremely stable, retaining 95% activity after storage for 30 days at 37°C with concentrations as low as 0.5 µg/mL.

## Package Contents and Storage Conditions

- **25X IgG Activator:** Store at -20°C upon arrival. Keep the vial in the desiccated container.
- **RPE-Z™:** Store at 2-8°C upon arrival. **Do not allow it to freeze!**
- **1X Quenching Reagent:** Store at -20°C ~ 8°C upon arrival. Does not need to be desiccated.

## Features

- Liquid-based reagents
- Completely scale-able: conjugate anywhere from 0.1 to 1 gram IgG per reaction.
- Supplies sufficient activated RPE to conjugate all IgG at a 1:1 RPE:IgG molar ratio.
- Highly efficient RPE incorporation - purification not usually necessary.
- Customize the RPE:IgG ratio to create optimized conjugates for different applications.
- Conjugates have greatly improved stability vs Lightning-Link™ and other chemistries.

## Products and Contents

Catalog Number	AR100080	AR100081	AR100082	AR100083	AR100084
For Labeling:	0.2 mg IgG	1 mg IgG	5 mg IgG	10 mg IgG	100 mg IgG
25X IgG Activator	10 µL	10 µL	10 µL	10 µL	20 µL
RPE-Z™ (20 mg/mL)	16 µL	80 µL	400 µL	0.80 mL	8.0 mL
Activated RPE	0.32 mg	1.6 mg	8 mg	16 mg	160 mg
1X Quenching Reagent	25 µL	25 µL	60 µL	120 µL	1000 µL

## Additional Reagents Required

1X Phosphate Buffered Saline (1X PBS), pH 7.2-7.4

Deionized water

Desalting columns

## Shelf Life

The performance of the product is guaranteed for a minimum of 12 months when stored as directed.

## IgG Amount and Concentration and Buffers

The IgG to be labeled should be at a concentration 1.0 -10.0 mg/ml in pure 1X PBS and should not contain any preservatives or carriers such as sodium azide, Proclin 300 or BSA.

## RPE:IgG Molar Ratio

The recommended RPE:IgG molar ratio for an initial conjugation reaction is 1:1\*. However, lower or higher ratios may give better results depending upon the antibody characteristics and the intended end-use. Conjugates for bead-based immunoassay platforms may perform optimally at a different RPE:IgG molar ratio than conjugates to be used for flow cytometry.

The table below shows the conversion from molar ratio to mass ratio and the volume of activated RPE required per mg of IgG for each molar ratio.

RPE:IgG Molar Ratio	RPE:IgG Mass Ratio	Vol. Activated RPE (RPE-Z™) per mg of IgG
0.50 : 1	0.80 : 1	40 µL
0.75 : 1	1.20 : 1	60 µL
1.00 : 1*	1.6 : 1	80 µL
1.25 : 1	2.0 : 1	100 µL
1.50 : 1	2.4 : 1	120 µL

## Experimental Protocols

1. Desalt IgG into 1X PBS, pH 7.2 – 7.4. To quantitate IgG, divide the A280 by 1.4 to give the IgG concentration in mg/mL.

2. Calculate volume of 1X IgG Activator required: 2 µL of 1X IgG Activator is required per mg of IgG. For small amounts of IgG, IgG Activator can be diluted an additional 10-fold (to 0.1X). In this case, 20 µL of 0.1 X IgG Activator solution is required per mg of IgG.

Note: Diluted (1X or 0.1X) IgG Activator must be used within 5 minutes of preparation. If more than 5 minutes passes before use, discard the solution and prepare a fresh solution.

3. Remove the 25X IgG Activator from the freezer. Allow it to equilibrate to room temperature before opening the outer vial.

4. Prepare 1X diluted IgG Activator from 25X IgG Activator in deionized water:
    - a. 2  $\mu$ L of 1X Activator is required per mg of IgG.
    - b. Measure at least 1 mg of the 25X IgG Activator by weight on an accurate analytical balance, using an appropriate pipettor with a disposable tip to deliver the liquid into a tared Eppendorf or comparable tube.
    - c. Add 24  $\mu$ L of dH<sub>2</sub>O to each mg of Activator weighed out.
    - d. Immediately vortex to mix the activator thoroughly.
  5. Add 2  $\mu$ L of 1X Activator per mg of IgG to the IgG solution (or 20  $\mu$ L of 0.1X Activator per mg of IgG).
  6. Incubate the solution at room temperature for 1 hour with mixing or shaking. A longer incubation is not harmful and even overnight incubations will be successful.
  7. Desalt the IgG into pure 1X PBS.
    - a. We recommend Pierce Zeba desalting spin columns with a 7 Kd MW cutoff for small volumes of IgG.
    - b. Gravity columns are acceptable for larger volumes.
    - c. Note: Activated IgG may be used without desalting, but the resulting conjugate may not perform as well.
  8. Quantitate the concentration and amount of activated IgG. The IgG concentration should be greater than 0.5 mg/mL.
- Note: The activated IgG is stable and can be stored at 2-8°C for up to 1 month.**
9. Calculate the volume of RPE-Z™ required for your desired RPE:IgG ratio (see table under RPE:IgG Molar Ratio)
  10. Add the calculated volume of RPE-Z™ to the IgG solution.
  11. Mix gently at room temperature for 18-24 hours. End-over-end mixing is ideal, but other types of gentle mixers or shakers can be used.
  12. Remove the Quenching Reagent from the freezer. Allow it to reach room temperature before opening the vial.
  13. Add 5  $\mu$ L of Quenching Reagent per mg of RPE-Z™ to the reaction.

14. Mix at room temperature for 1 hour or longer. A longer incubation is not harmful and overnight incubations are fine.

15. Test conjugate in the desired application. To improve performance, purify the conjugate by size exclusion chromatography.

## RECOMMENDED ACCESSORIES

**For desalting IgG after activation - Order from ThermoFisher :**

Sample Size	Description	Cat #
2 – 12 $\mu$ L	Zeba Spin Desalting Columns, Micro (75 $\mu$ L), 7K MWCO	89877, 89878
30 - 130 $\mu$ L	Zeba Spin Desalting Columns, 0.5 mL, 7K MWCO	89882, 89883
200 – 700 $\mu$ L	Zeba Spin Desalting Columns, 2 mL, 7K MWCO	89889, 89890
500 – 2000 $\mu$ L	Zeba Spin Desalting Columns, 5 mL, 7K MWCO	89891, 89892
700 – 4000 $\mu$ L	Zeba Spin Desalting Columns, 10 mL, 7K MWCO	89893, 89894

**For concentrating IgG before or after activation or for concentrating the final conjugate – Order from MilliporeSigma:**

Sample Size	Description	Cat #
Up to 500 $\mu$ L	Amicon Ultra-0.5 Centrifugal Filter Unit with Ultracel-50 membrane	Z740176
Up to 2 mL	Amicon Ultra-2 Centrifugal Filter Unit with Ultracel-50 membrane	UFC205024
Up to 4 mL	Amicon Ultra-4 Centrifugal Filter Unit with Ultracel-50 membrane	UFC805008
Up to 15 mL	Amicon Ultra-15 Centrifugal Filter Unit with Ultracel-50 membrane	Z648000