

# RPE-IgG Conjugation Kit

## 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

Kit Component	Storage Temp	Storage Notes
Concentrated Activator	-20°C	Keep the vial in the desiccated container as supplied in the kit
RPE-Z™ - Activated RPE (20mg/ml)	-20°C or 2-8°C	Does not need to be kept desiccated.
1x Quenching Reagent	-20°C or 2-8°C	Does not need to be kept desiccated.

## Features

- Liquid-based reagents.
- Completely scalable: conjugate anywhere from 10 µg to 1 gram IgG per reaction.
- Supplies sufficient activated RPE to conjugate all IgG at a 1:1 RPE:IgG 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

<b>Catalog Number</b>	<b>AR100082</b>
<b>For Labeling:</b>	<b>1 x 5 mg IgG</b>
Concentrated Activator	10 $\mu$ L
RPE-Z™ Activated RPE (20 mg/mL)	400 $\mu$ L
1x Quenching Reagent	80 $\mu$ L

## Additional Reagents Required But Not Supplied

- 1X Phosphate Buffered Saline (1X PBS), pH 7.2-7.5
- Deionized water (dH<sub>2</sub>O)
- 1.5 mL microcentrifuge tubes

## Shelf Life

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

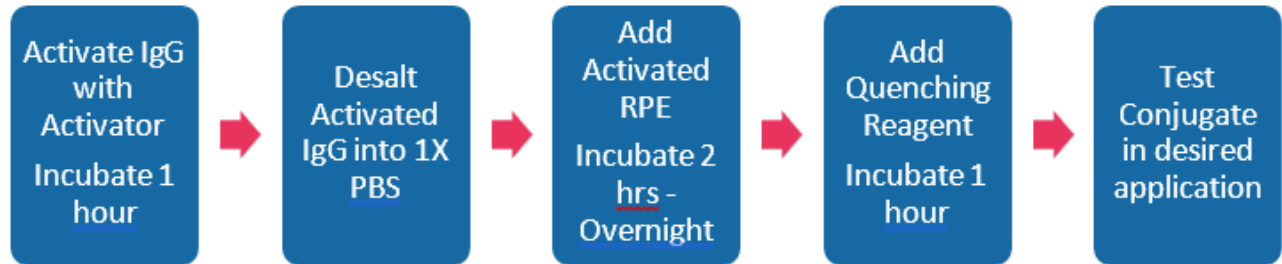
## IgG Requirements

The IgG to be labeled should be at a minimum concentration of 0.8 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

This kit utilizes a 1:1 RPE:IgG molar ratio which is optimal for most conjugations reaction. However, lower or higher ratios may give better results depending upon the antibody characteristics and the intended end-use. To change the RPE:IgG molar ratio, vary the volume of RPE-Z™ added to the conjugation reaction.

## Conjugation Procedure - Overview



## Before Beginning the Procedure

Remove the Concentrated Activator from the freezer. Important: Allow sufficient time to let the container and contents come to room temperature before opening the outer and inner vials.

Note: The jar containing the Activator can be removed from the freezer up to 24 hours before use.

## Detailed Conjugation Procedure

1. Measure the absorbance of the IgG solution at 280 nm using PBS as a blank. Divide the A<sub>280</sub> by 1.40 to obtain the IgG concentration in mg/ml.
2. Dilute IgG to 1.20 mg/ml in 1X PBS (0.80 – 1.4 mg/ml is acceptable).
3. Add 50 mg of IgG solution to a new microcentrifuge tube.
4. Prepare a working dilution (1X) of Activator from Concentrated Activator in deionized water:
  - a. Add 2.0 µL of Concentrated Activator to 60 µL of deionized water.
  - b. Immediately vortex to mix the solution thoroughly.

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

5. Add 50 µL of 1X Activator to 5 mg aliquot of IgG and then mix thoroughly by gently vortexing.
6. Incubate the solution at room temperature for 1 hour.

Note: A longer incubation is not harmful and overnight incubations will be successful.

7. Desalt the complete reaction volume into pure 1X PBS. We recommend using Pierce Zeba desalting spin columns with a 7 Kd MW cutoff for small volumes of IgG. Use of gravity desalting columns, dialysis, and extensive washing with centrifugal filter units for desalting is also acceptable.

Note: The activated IgG is stable and can be stored at 2-8°C for at least 4 months.

8. Add 400 µL of RPE-Z™ to the desalted, activated IgG and mix by gentle vortexing.
9. Incubate the solution at room temperature for 2-24 hours.

Note: Usable conjugates are produced after only 2 hours of conjugation. Larger and more potent conjugates will be produced after longer incubations.

10. Add 80 µL of Quenching Reagent to the reaction and mix by gently vortexing.

11. Incubate the solution at room temperature for 1 hour.

Note: A longer incubation is not harmful and overnight incubations will be successful.

12. Conjugate is ready for use. Store at 2-8°C.

Note: To improve conjugate performance, it may help to purify the conjugate from the unincorporated RPE and reaction components by size exclusion chromatography.

## OPTIONAL ACCESSORIES

**For desalting IgG before activation - Order from Thermo Fisher Scientific:**

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

**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

