

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

Kit Component	Storage Temp	Storage Notes
Concentrated Activator	-20°C	Keep the vial in the desiccated container as supplied in the kit
RPE-Z™	-20°C or 2-8°C	Does not need to be kept desiccated.
Quenching Reagent	-20°C or 2-8°C	Does not need to be kept desiccated.
Zeba Desalting Column with Collection Tube	2-8°C	Does not need to be kept desiccated. Do not store at freezing temperatures.

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	3x 100ug IgG	5 mg IgG	10 mg IgG	100 mg IgG
Concentrated Activator	10 µL	10 µL	10 µL	10 µL	20 µL
RPE-Z™ (16 mg/mL)	16 µL	36 µL	400 µL	0.80 mL	8.0 mL
Zeba Desalting Column	0.32 mg	3 each	8 mg	16 mg	160 mg
Quenching Reagent	25 µL	40 µL	60 µL	120 µL	1000 µL

Additional Reagents Required But Not Supplied

- 1X Phosphate Buffered Saline (1X PBS)
- pH 7.2-7.5 Deionized water (dH2O)
- ml microcentrifuge tubes

Shelf Life

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

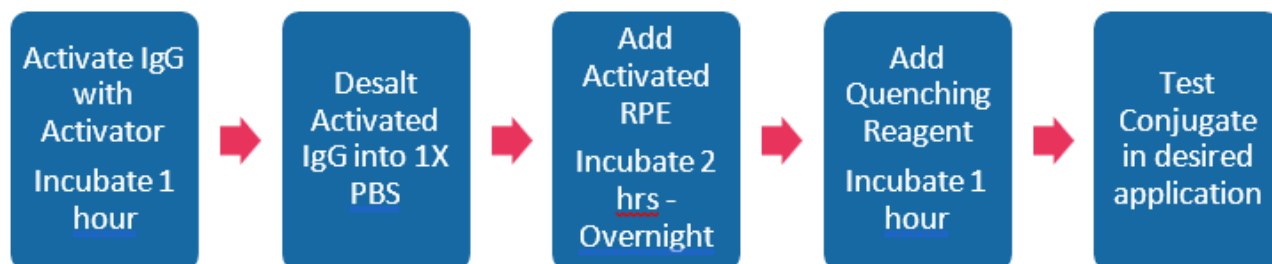
IgG Requirements

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 A₂₈₀ 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 100 μ L 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 μ L of Concentrated Activator to 1300 μ L 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 pass before use, discard the 1X Activator and prepare a fresh solution.

- 5. Add 2.0 μ L of 1X Activator to the 10 μ L aliquot of IgG and then mix thoroughly by gentle 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 12 μ L reaction volume into pure 1X PBS using the included Zeba spin column. See the attached desalting protocol.**

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

- 8. Add 2 μ L 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 2 μ L of Quenching Reagent to the reaction and mix by gentle 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. Test conjugate in the desired application.

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

RECOMMENDED ACCESSORIES

For desalting IgG after activation - Order from ThermoFisher :

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