

Instructions for Use

RPE-IgG Conjugation Kit

Conjugation kit for the preparation of stable and reproducible RPE-antibody conjugates for enzymatic labeling in immunochemistry.



CNJ93101



Read the documentation

For Research Use Only



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The Native Antigen Company is now part of LGC Group.

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REAGENT STORAGE

Note mixed storage requirements

Store the 25X IgG Activator at -20°C. Keep the vial in the desiccated container as supplied in the kit.

Store RPE-Z™ at -20°C to 8°C. Does not need to be kept desiccated.

Store 1X Quenching Reagent at -20°C to 8°C. Does not need to be kept desiccated.

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 and multiplex immunoassays. 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 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° with concentrations as low as 0.5 µg/mL.

FEATURES

- Liquid-based reagents
- Completely scaleable: conjugate anywhere from 25 µg 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	CNJ 93101-0.20	CNJ 93101-001	CNJ 93101-005
For Labeling:	0.2 mg IgG	1 mg IgG	5 mg IgG
25X IgG Activator	10 µL	10 µL	10 µL
RPE-Z™ (20 mg/mL) Activated RPE	16 µL 0.32 mg	80 µL 1.6 mg	400 µL 8 mg
1X Quenching Reagent	25 µL	25 µL	60 µL

ADDITIONAL REAGENTS REQUIRED BUT NOT SUPPLIED

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 0.8 - 4.0 mg/ml in pure 1X PBS and should not contain any preservatives or carriers such as sodium azide, Proclin 300 or BSA.

PE: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

REAGENT AMOUNTS FOR A 1:1 RPE:IgG CONJUGATION REACTION

IgG Amount	Activator Concentration	Activator Volume	RPE-Z™ Volume	Quenching Rgt Vol.
25 µg	0.1X	1 µL	2 µL	1.0 µL
50 µg	0.1X	2 µL	4 µL	1.0 µL
100 µg	0.1X	4 µL	8 µL	1.6 µL
200 µg	0.1X	8 µL	16 µL	3.2 µL
250 µg	0.1X	10 µL	20 µL	4.0 µL
500 µg	0.1X	20 µL	40 µL	8.0 µL
0.5 mg	1X	2 µL	40 µL	8 µL
1.0 mg	1X	4 µL	80 µL	16 µL
2.0 mg	1X	8 µL	160 µL	32 µL
2.5 mg	1X	10 µL	200 µL	40 µL
5.0 mg	1X	20 µL	400 µL	80 µL
10.0 mg	1X	40 µL	800 µL	160 µL

CONJUGATION PROCEDURE FOR 25-500 µg IgG

1. If necessary, desalt IgG into 1X PBS, pH 7.2 – 7.4. 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. Calculate the volume of 0.1X IgG Activator required: 0.04 µL of 0.1X IgG Activator solution is required per µg of IgG.
3. Remove the 25X IgG 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 vial containing the IgG Activator can be removed from the freezer up to 24 hours before use.

4. Prepare 0.1X diluted IgG Activator from 25X IgG Activator in deionized water:
 - a. 0.04 μL of 0.1X Activator is required per μg 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 250 μL of dH₂O for each mg of Activator weighed out.
 - d. Immediately vortex to mix the solution thoroughly.

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

5. Add 0.04 μL of 0.1X Activator to IgG and then mix thoroughly by gentle vortexing.
6. Incubate the solution at room temperature for 1 hour. A longer incubation is not harmful and even overnight incubations will be successful.
7. Desalt the IgG into pure 1X PBS. We recommend 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.
8. Quantitate the concentration and amount of activated IgG. The activated IgG concentration should be greater than 0.8 mg/mL.

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

9. Calculate the volume of RPE-ZTM required for your desired RPE:IgG ratio (see table under RPE:IgG Molar Ratio)
10. Add the calculated volume of RPE-ZTM to the desalted IgG solution and mix by gentle vortexing.
11. Incubate the solution at room temperature for 2-24 hours. Usable conjugates are produced after only 2 hours of conjugation. However, ideal conjugates will be produced after an overnight incubation.
12. Add 0.2 μL of Quenching Reagent per μL of RPE-ZTM added to the reaction. For reactions requiring less than 1 μL Quenching Reagent, add 1 μL Quenching Reagent to the reaction.
13. Incubate the solution at room temperature for 1 hour. A longer incubation is not harmful and overnight incubations are fine.
14. Test conjugate in the desired application. To improve performance, purify the conjugate from the unincorporated RPE and reaction components by size exclusion chromatography.

STORAGE

RPE-IgG Conjugate can be stored at 4°C. For longer storage periods, adding 10% BSA is acceptable.

CONJUGATION PROCEDURE FOR 0.5-10 mg IgG

1. If necessary, desalt IgG into 1X PBS, pH 7.2 – 7.4. 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. Calculate the volume of 1X IgG Activator required: 4 μ L of 1X IgG Activator solution is required per mg of IgG.
3. Remove the 25X IgG Activator from the freezer. **Important:** Allow sufficient time to allow the container and contents to come to room temperature before opening the outer and inner vials.

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

4. Prepare 1X diluted IgG Activator from 25X IgG Activator in deionized water:
 - a. 4 μ L of 1X Activator is required per μ g 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 μ L of dH₂O for each mg of Activator weighed out.
 - d. Immediately vortex to mix the activator thoroughly.

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

5. Add 4.0 μ L of 1X Activator per mg of IgG and then mix thoroughly by gentle vortexing.
6. Incubate the solution at room temperature for 1 hour. A longer incubation is not harmful and even overnight incubations will be successful.
7. Desalt the IgG into pure 1X PBS. We recommend Pierce Zeba desalting spin columns with a 7 Kd MW cutoff for small volumes of IgG. Use of gravity desalting columns and extensive washing with centrifugal filter units is also acceptable.
8. Quantitate the concentration and amount of activated IgG. The IgG concentration should be greater than 0.8 mg/mL.

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

9. Calculate the volume of RPE-ZTM required for your desired RPE:IgG ratio (see table under RPE:IgG Molar Ratio)
10. Add the calculated volume of RPE-ZTM to the IgG solution and mix by gentle vortexing.
11. Incubate the solution at room temperature for 2-24 hours. Usable conjugates are produced after only 2 hours. However, ideal conjugates will be produced after an overnight incubation.
12. Add 0.2 μ L of Quenching Reagent per μ L of RPE-ZTM added to the reaction. Incubate the solution at room temperature for 1 hour. A longer incubation is not harmful and overnight

incubations are fine.

13. Test conjugate in the desired application. To improve performance, purify the conjugate from the unincorporated RPE and reaction components by size exclusion chromatography.

STORAGE

RPE-IgG Conjugate can be stored at 4°C. For longer storage periods, adding 10% BSA is acceptable.

RECOMMENDED ACCESSORIES

For desalting IgG after activation - Order from ThermoFisher :

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