HRP-IgG Conjugation Kit

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Introduction

Horseradish Peroxidase is widely used as an enzymatic label in immunochemistry assays such as ELISA. Preparing stable and reproducible antibody-HRP conjugates is one of the biggest challenges of developing immunoassays. The OriGene HRP-IgG conjugation utilizes a novel chemistry to generate highly reproducible IgG-HRP conjugates with a simple procedure. The resulting conjugates have been shown to be extremely stable, retaining 94% activity after storage for 95 days at 37º when stored at a concentration of 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.
- **HRP-Z™:** Store at -20°C ~ 8°C upon arrival. Does not need to be kept desiccated.
- **1X Quenching Reagent:** Store at -20°C ~ 8°C upon arrival. Does not need to be kept desiccated.

Features

- Liquid-based reagents.
- Completely scalable: conjugate anywhere from 0.1 to 1 gram IgG per reaction.
- Supplies sufficient activated HRP to conjugate all IgG at a 4:1 HRP:IgG ratio.
- Highly efficient HRP incorporation - purification not usually necessary.
- Customize the HRP:IgG ratio to create optimized conjugates for different applications.
- Conjugates have greatly improved stability vs Lightning-Link™ and traditional chemistry.

Products and Contents

Catalog Number	AR100075	AR100076	AR100077	AR100078	AR100079
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
HRP-Z™ - Activated	12 µL	60 µL	300 µL	600 µL	6.0 mL
HRP (20 mg/mL)	0.24 mg	1.2 mg	6 mg	12 mg	120 mg
1X Quenching Reagent	25 µL	25 µL	60 µL	120 µL	1.2 mL

Additional Reagents Required

1X Phosphate Buffered Saline (1X PBS), pH 7.2-7.5

Deionized water (dH2O)

Desalting columns (see Accessories section)

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.

HRP:IgG Molar Ratio

The recommended HRP:IgG molar ratio for most conjugations reaction is 4:1. However, lower or higher ratios may give better results depending upon the antibody characteristics and the intended end-use. Conjugates for ELISA may perform optimally at a different HRP:IgG molar ratio than conjugates to be used for immunohistochemistry.

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

HRP:IgG Molar Ratio	HRP:IgG Mass Ratio	Vol. Activated HRP per mg of IgG
1 : 1	0.3 : 1	15 µL
2 : 1	0.6 : 1	30 µL
3 : 1	0.9 : 1	45 µL
4 : 1 (recommended)	1.2 : 1	60 µL
5 : 1	1.5 : 1	75 µL
6 : 1	1.8 : 1	90 µL
8 : 1	2.4 : 1	120 µL

CONJUGATION PROCEDURE for less than 1 mg of IgG

1. 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 volume of 0.1X IgG Activator required: 40 uL of 0.1X IgG Activator solution is required per mg of IgG. **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.

3. Remove the 25X IgG Activator from the freezer. Allow sufficient time to allow the container and contents to come to room temperature before opening the outer vial. **Note:** The vial containing the IgG Activator can be removed from the freezer up to 24 hours before use.

4. Dilute IgG Activator to 0.1X in deionized water: 40 uL of 0.1X Activator is required per mg of IgG

5. Measure the IgG Activator by weight. To prepare 0.1X IgG Activator, add 250 uL of dH2O to each mg of IgG Activator weighed out.

6. Immediately vortex to mix the activator thoroughly and then add 40 uL of 0.1X Activator per mg of IgG.

7. Incubate the solution at room temperature for 1 hour with gentle mixing or shaking.

a. End-over-end mixing is ideal, but other types of gentle mixers or shakers can be used.

b. A longer incubation is not harmful and even overnight incubations will be successful.

8. 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.

9. 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 2-8ºC for at least 1 month.

10. Calculate the volume of HRP-Z™ required for your desired HRP:IgG ratio (see table under HRP:IgG Molar Ratio)

11. Add the calculated volume of HRP-Z™ to the IgG solution.

12. 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.

13. Remove the Quenching Reagent from the freezer. Allow it to reach room temperature before opening the vial.

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

14. Add 0.2 uL of Quenching Reagent per uL of HRP-Z™ added to the reaction.

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

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

CONJUGATION PROCEDURE for 1 mg or more of IgG

1. 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 volume of 1X IgG Activator required: 2 uL of 1X IgG Activator is required per mg of IgG. **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.

3. Remove the 25X IgG Activator from the freezer. Allow sufficient time to allow the container and contents to come to room temperature before opening the outer vial. Note: The vial containing the IgG Activator can be removed from the freezer up to 24 hours before use.

4. Dilute IgG Activator to 1X in deionized water: 2 uL of 1X Activator is required per mg of IgG

5. Measure the IgG Activator by weight. To prepare 1X Activator, add 25 uL of dH2O to each mg of IgG Activator weighed out.

6. Immediately vortex to mix the activator thoroughly and then add 2 uL of 1X Activator per mg of IgG to the IgG solution.

7. Incubate the solution at room temperature for 1 hour with gentle mixing or shaking.

   a. End-over-end mixing is ideal, but other types of gentle mixers or shakers can be used.

   b. A longer incubation is not harmful and even overnight incubations will be successful.

8. 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.

9. 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 2-8ºC for at least 1 month.

10. Calculate the volume of HRP-Z™ required for your desired HRP:IgG ratio (see table under HRP:IgG Molar Ratio)

11. Add the calculated volume of HRP-Z™ to the IgG solution.

12. 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.

13. Remove the Quenching Reagent from the freezer. Allow it to reach room temperature before opening the vial. Note: The vial containing the Quenching Reagent can be removed from the freezer up to 24 hours before use.

14. Add 0.2 uL of Quenching Reagent per uL of HRP-Z™ added to the reaction.

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

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

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