HRP-IgG Conjugation Kit

For labeling 3 x 10 µg IgG

Reagent Storage

The kit is shipped on blue ice. Please store kit components as described below

Kit Component	Storage Temp	Storage Notes
Concentrated Activator	-20°C	Keep the vial in the desiccated container as supplied in the kit
HRP-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 Columns with Collection Tubes	2-8°C	Does not need to be kept desiccated. Do not store at freezing temperatures

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 HRP-IgG conjugation kit utilizes a highly robust 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 37° when stored at a concentration of 0.5 µg/mL.

Features

- Liquid-based reagents.
- Completely scalable: conjugate anywhere from 10 µg 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.

Product & Contents

Catalogue Number	HRP-Link-CA
For Labeling:	3 x 10 µg IgG
Concentrated Activator	10 µL
HRP-Z™ - Activated HRP (6 mg/ml)	12 µL
1X Quenching Reagent	25 µL
Zeba Desalting Column with Collection Tube	3 each

Additional Reagents Required But Not Supplied

1X Phosphate Buffered Saline (1X PBS), pH 7.2-7.5
Deionized water (dH_2O)
1.5 ml microcentrifuge tubes

Shelf Life

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

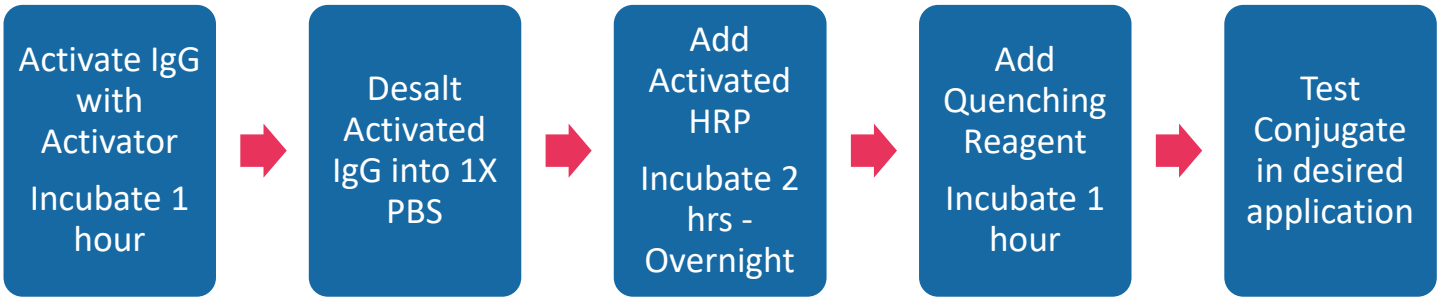
IgG Requirements

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.

HRP:IgG Molar Ratio

This kit utilizes a 4:1 HRP: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 HRP:IgG molar ratio, vary the volume of HRP-Z™ added to the conjugation reaction (Step 8).

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 (0.8 – 1.4 mg/ml is acceptable).
3. Add 10 µL 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 uL of Concentrated Activator to 1300 µL of deionized water (1:650 dilution).
    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 2.0 uL of 1X Activator to the 10 µL 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 even overnight incubations will be successful.

7. Desalt the complete 12 µL 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 µL of HRP-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 incubation. Larger and more potent conjugates will be produced after longer incubations.

10. Add 2 µL 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 HRP 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 µL	Zeba Spin Desalting Columns, Micro (75µL), 7K MWCO	89877, 89878
30 - 130 µL	Zeba Spin Desalting Columns, 0.5mL, 7K MWCO	89882, 89883

For concentrating IgG before or after IgG 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

Zeba Spin Desalting Column Instructions

75 µL columns for 2 – 12µL Samples

Notes:

- Each column can desalt a 2-12 µL sample.
- The resin slurry contains 0.03% sodium azide.
- These columns are recommended for desalting molecules > 7000 Daltons.

Storage:

- Store columns at 2-8°C.
- Columns may be stored at room temperature for several days without adverse effects.

Additional Materials Required

- Variable-speed bench-top microcentrifuge
- 1.5 mL microcentrifuge collection tubes

Spin Column Preparation

1. Remove column's bottom closure and loosen cap (do not remove cap).
2. Place column in the collection tube provided.
3. Centrifuge at 1000 × *g* for 1 minute to remove storage solution.
4. Discard storage solution from the collection tube. Remove column cap. It is not necessary to replace the cap for subsequent steps.
5. Add 50 µL of buffer on top of the resin bed. Centrifuge at 1000 × g for 1 minute to remove buffer. Discard the buffer from the collection tube.
6. Repeat Steps 5 two additional times for a total of 3 column washes.

Sample Desalting

7. Place column in a new microcentrifuge tube and slowly apply the sample to the center of the compacted resin bed.
8. Do not add a stacker after application of the sample to the resin bed.
9. Centrifuge column at 1000 × g for 2 minutes to collect desalted sample. Discard desalting column after use.

Zeba Desalting Columns are a product of Thermo Fisher Scientific Inc.

Sample Size	Description	Cat #
2 – 12 µL	Zeba Spin Desalting Columns, Micro (75µL), 7K MWCO	89877, 89878