HRP-IgG Conjugation Kit

For labeling 1 x 1 mg 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	-20ºC	Keep the vial in the desiccated container as supplied in the kit
HRP-Z™	-20ºC or 2-8ºC	Does not need to be kept desiccated.
Quenching Reagent	-20ºC or 2-8ºC	Does not need to be kept desiccated.

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 37º 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 and CONTENTS

Catalog Number	**HRP-Link-AF**
For Labeling:	**1 x 1 mg IgG**
Concentrated Activator	10 µL
HRP-Z™ - Activated HRP (20 mg/ml)	60 µL
1X Quenching Reagent	25 µL

ADDITIONAL REAGENTS REQUIRED BUT NOT SUPPLIED

1X Phosphate Buffered Saline (1X PBS), pH 7.2-7.5
Deionized water (dH_2O)
Desalting columns (see Accessories section)

SHELF LIFE

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

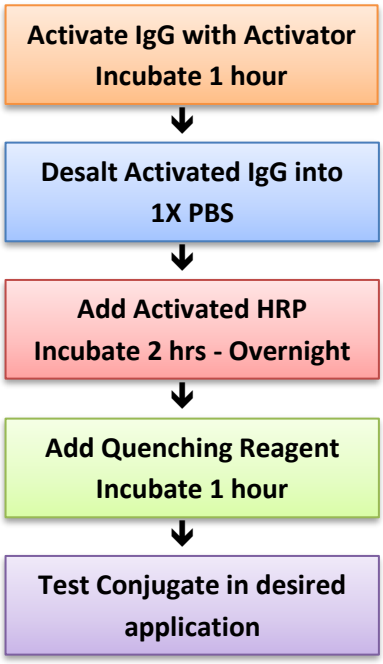
IgG Requirements

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

Note: This protocol can be split up to make multiple smaller sized reactions. The reaction volumes of steps 5 and 8 are directly proportional to the amount of starting material from step 1.

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 1.0 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 10.0 µL of 1X Activator to the 1 mg 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 reaction volume 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.

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

8. Add 60 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 12 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. 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 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, 0.5mL, 7K MWCO	89882, 89883
200 – 700 µL	Zeba Spin Desalting Columns, 2mL, 7K MWCO	89889, 89890
500 – 2000 µL	Zeba Spin Desalting Columns, 5mL, 7K MWCO	89891, 89892
700 – 4000 µL	Zeba Spin Desalting Columns, 10mL, 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