

HRP-IgG Conjugation Kit

For labeling 1 x 10 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	-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.

INTRODUCTION

Horseshradish 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 and CONTENTS

Catalog Number	CNJ93100-010
For Labeling:	1 x 10 mg IgG
Concentrated Activator	10 µL
HRP-Z™ - Activated HRP (20 mg/ml)	600 µL
1X Quenching Reagent	120 µL

ADDITIONAL REAGENTS REQUIRED BUT NOT SUPPLIED

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.

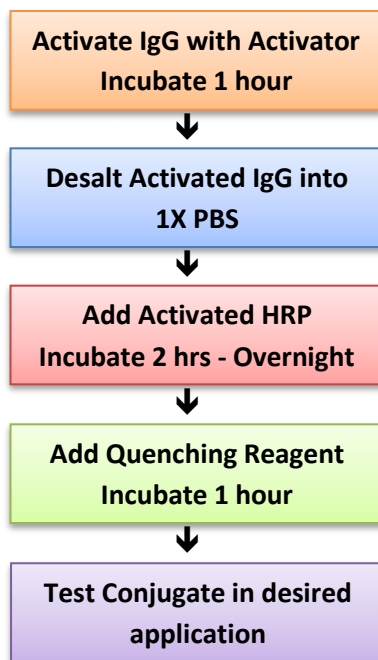
IgG Requirements

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 the 10 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 4.0 μ L of Concentrated Activator to 120 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 passes before use, discard the 1X Activator and prepare a fresh solution.

5. Add 100 μ L of 1X Activator to the 10 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 2-8°C for at least 4 months.

8. Add 600 μ L 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 120 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. 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