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HRP Conjugation Kit -

For simple and quick conjugation of IgG antibodies with HRP. For research use only - not intended for diagnostic use.

Introduction:

HRP Conjugation Kit - provides a simple and rapid procedure to covalent labeling of antibodies with HRP by targeting primary amine groups (e.g. lysines). It takes only few mins hands-on time and conjugates are ready to use in less than 3 hours without loss of antibodies.
The antibody to be labeled should be purified, in an appropriate buffer for conjugation and at a suitable concentration conjugation (as described in buffer considerations).
The kit comes in 5 pack sizes:

- 3 x 10 µg containing 3 reactions (each for 10 - 20 µg)
- 100 µg containing 1 reaction (for 100 - 200 µg)
- 3 x 100 µg containing 3 reactions (each for 100 - 200 µg)
- 1 mg containing 1 reaction (for 1 - 2 mg)
- 3 x 1 mg containing 3 reactions (each for 1 - 2 mg)

Custom size conjugation kits up to 100 mg are available on demand. Please contact us to discuss your requirements.

Materials Supplied:

Item	3 x 10 µg	100 µg	3 x 100 µg	1 mg	3 x 1 mg	Storage temperature
HRP mix	3 vials	1 vial	3 vials	1 vial	3 vials	-20°C
Modifier reagent	1 vial	1 vial	1 vial	1 vial	1 vial	-20°C
Quencher reagent	1 vial	1 vial	1 vial	1 vial	1 vial	-20°C

Storage and Stability: Store kit at -20ºC immediately upon receipt.

Buffer considerations:

Recommended pre-conjugation buffer components and conditions:

Buffer Components & Conditions	
Purified antibody	Yes
Antibody in ascites fluid, serum, hybridoma or tissue culture media	No
Antibody concentration	1-8 mg/mL
pH	6.5-8.5
Amine free buffer (e.g. MES, MOPS, HEPES, PBS)	Yes
Non-buffering salts (e.g. sodium chloride)	Yes
BSA	No
Sodium Azide	No
Chelating agents (e.g. EDTA)	Yes
Glycerol	<20%
Sugars	Yes
Gelatin[1]	<0.05%
Tris	<50mM
Glycine	No
Thiomersal / Thimerosal	No
Merthiolate	No
Proclin	No
Borate buffer	Yes
Nucleophilic components (Primary amines e.g. amino acids or ethanolamine and thiols e.g. mercaptoethanol or DTT)	No

Recommended starting amount and volume of antibody:

Vial Size	Lowest Amount of Antibody	Recommended Amount of Antibody (1:2 Antibody:HRP)	Maximum Amount of Antibody (1:1 Antibody:HRP)	Fixed Volume of Antibody
10 µg	Down to 10 µg at 1 mg/mL	Optimally 10 µg at 4 mg/mL	Up to 20 µg at 4 mg/mL	Fixed 10 µL
100 µg	Down to 100 µg at 1 mg/mL	Optimally 100 µg at 4 mg/mL	Up to 200 µg at 4 mg/mL	Fixed 100 µL
1 mg	Down to 1mg at 1 mg/mL	Optimally 1,000 µg at 4 mg/mL	Up to 2,000 µg at 4 mg/mL	Fixed 1,000 µL

***Note**: It is important to use the indicated fixed volumes. Modifying the reaction volume may results in poor quality conjugates.*

***Note**: Antibodies can be diluted using either milli-Q water or PBS.*

***Note**: Optimal conjugates are normally generated using the recommended amount of antibody, although using different amounts of antibody within the range provided will still generate quality conjugates.*

***Note**: Adding less than the recommended amount of antibody may result in unbound label post conjugation. This excess label will be deactivated by the quencher and removed during the first wash step of any application.*

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.

1. Add 1 µL of Modifier reagent to each 10 µL of antibody to be labeled and mix gently.
2. Remove cap from vial of HRP Conjugation Mix and pipette the antibody sample (with added Modifier reagent) directly onto the material. Resuspend gently by withdrawing and re-dispensing the liquid once or twice using a pipette.
3. Replace cap on the vial and leave standing for 3 hours in the dark at room temperature (20-25°C) or overnight at 4°C.
4. After incubating for 3 hours (or more), add 1 µL of Quencher reagent for every 10 µL of antibody used and mix gently. The conjugate can be used after 30 minutes. The conjugates do not require purification.

Conjugate Storage

Typically, the antibody conjugate can be stored at 4°C for up to 12 months. For longer storage the conjugate can be stored at -20°C with a cryoprotectant such as 50% glycerol. The best storage conditions for any particular conjugate must be determined by experimentation and depend on the antibody stability and best storage conditions. The antibody conjugate should always be stored in the dark.