

Horseradish Peroxidase (HRP)

H1508159

Storage: -20°C. Avoid freeze/thaw cycle. Store in the dark. Desiccated.

Introduction:

Horseradish Peroxidase (HRP) is a hemoglobin with redox properties, a common reagent in clinical chemistry. It can conjugate with antibodies, antigens, or streptavidin, and is widely used in clinical diagnosis, immunoassays, chemiluminescence, ELISA, immunohistochemistry, and biochemical research.

Basic Product Information:

1. Name: Horseradish Peroxidase (HRP).
2. Source: Extracted from the roots of *Armoracia rusticana* (horseradish).
3. Molecular weight: Approximately 40 kDa (single-chain polypeptide, containing 4 disulfide bonds).
4. Activity unit: ≥ 300 units/mg.
5. Purity index: RZ value (Reinheit Zahl) ≥ 3.0 (reflects the purity ratio of hemoglobin to enzyme protein).
6. Storage conditions: Store at -20°C in a dry, dark place. After reconstitution, it remains stable for 6 weeks at 4°C; for long-term storage, aliquot and keep at -70°C.

Physicochemical Properties:

1. Appearance: Brown crystalline substance or lyophilized powder.
2. Solubility: Soluble in water; soluble below 58% ammonium sulfate saturation.
3. Stability:
 - pH range: 3.0-9.0 (isoelectric point).
 - Thermostability: Retains activity after heating at 63°C for 15 minutes.
4. Inhibitors: Sodium azide, cyanide, heavy metal ions (e.g., Cd^{2+} , Cu^{2+}).

Functions and Applications:

1. Catalytic mechanism: HRP catalyzes oxidation reactions involving hydrogen peroxide (H_2O_2), converting substrates (e.g., DAB, TMB) into chromogenic products.
2. Main application fields:

Application Type	Specific Uses	Example Methods
Detection Immunology	Antibody labeling for ELISA, Western Blot, Immunohistochemistry (IHC)	HRP-labeled secondary antibody + streptavidin
Molecular Biology	Probe labeling for nucleic acid hybridization (Southern/Northern Blot), EMSA	Biotin-streptavidin system
Biochemical Analysis	Quantitative detection of glucose peroxide	Colorimetry/chemiluminescence
Pathological Research	Neuron tracing (axoplasmic transport tracing)	HRP retrograde labeling

Usage Guidelines:

- Labeling operation (taking antibody labeling as an example).
 - Labeling methods: Glutaraldehyde cross-linking, periodate oxidation.
 - Recommended ratio: HRP:antibody = 1:1-1:3 (molar ratio).
 - Purification step: Remove free enzyme via gel filtration chromatography.
- Chromogenic systems:

Substrate	Chromogenic Product	Detection Method	Application Scenario
DAB	Brown precipitate	Light microscope/chemical imaging	Immunohistochemistry, Western Blot
TMB	Blue soluble substance (turns yellow after acidification)	Microplate reader (450 nm)	ELISA
ECL chemiluminescent reagent	Luminescent signal	X-ray film/ chemiluminescence imager	High-sensitivity Western Blot

Precautions:

- Avoid repeated freeze-thaw cycles: Aliquot and store at -70°C to prevent activity loss.
- Inhibitor interference: Avoid using buffers containing sodium azide (NaN₃).
- Safety protection: Wear gloves when handling DAB; dispose of waste liquid as hazardous chemical waste.
- Optimization suggestion: Adjust HRP concentration according to different applications (e.g., 1:2000-1:10000 dilution is recommended for Western Blot).