

**Amplite® West ECL HRP Substrate \*Femto Sensitivity\***Catalog number: 26100, 26101  
Unit size: 20 ml, 100 ml

Component	Storage	Amount (Cat No. 26100)	Amount (Cat No. 26101)
Component A: Solution A	Refrigerated (2-8 °C), Minimize light exposure	10 mL	50 mL
Component B: Solution B	Refrigerated (2-8 °C), Minimize light exposure	10 mL	50 mL

**OVERVIEW**

Amplite® West ECL HRP Substrate is an ultra-sensitive chemiluminescent (ECL) substrate formulated for highest sensitivity and stability. It is used for detection of peroxidase activity from HRP-conjugated antibodies and probes on nitrocellulose or PVDF membranes. The substrate provides a quick and efficient way for detecting proteins expressed at femtogram levels. It does not require any expensive instruments and consumables and is compatible with the existing instruments commonly used for western blots. When combined with very diluted and optimized antibody concentrations and blocking buffers, Amplite® West ECL HRP Substrate enables detection of target proteins in small amounts.

**AT A GLANCE****Protocol Summary**

1. Prepare working solution by mixing equal parts of Solution A and Solution B
2. Incubate membrane with working solution
3. Image with an imaging system or X-ray film

**SAMPLE EXPERIMENTAL PROTOCOL**

1. After incubating with the HRP enzyme conjugated antibody, wash the membrane 6 times for 5 minutes each in proper wash buffer to remove any unbound conjugates.
2. Prepare the developing working solution by mixing equal parts of Solution A and Solution B. **Note:** Use a sufficient volume to ensure the blot is completely wetted and will not dry out during development (0.1 mL/cm<sup>2</sup> )
3. Incubate the membrane with the working solution for 5 minutes.
4. Remove membrane from the working solution and wash briefly with wash buffer, remove excess liquid with an absorbent tissue if necessary.
5. Image the blot using an imaging system or X-ray film.

**DISCLAIMER**

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