

## Chemiluminescent Substrate Solution (HRP Marker)

**Cat. No. ABK-002      Size 100mL\*2**

### Description (Background)

The Chemiluminescent Substrate Solution (HRP Marker) is a specific triggering reagent for peroxidase labels such as horseradish peroxidase (HRP) and can be used in ELISA procedures. This substrate is an enhanced chemiluminescent substrate enables detection at femtogram range of target by oxidizing luminol in the presence of horseradish peroxidase (HRP) and peroxide.

This product is provided in two liquid buffer of Solution A (Luminol-Based Solution) and Solution B (Peroxide Solution). When used, mix Solution A and Solution B in equal volume by 1:1, and it gives a rapid light emission.

### Specifications

Items	Details
Detection Method	Chemiluminescence Enzyme Immunoassay (CLEIA)
Product Type	Chemiluminescent substrate for HRP labels
Contents	Solution A (Luminol-Based Solution) and Solution B (Peroxide Solution)
Quantity	100mL Solution A and 100mL Solution B in each kit
Appearance	Clear solution
Storage temperature	Stable for 1 year at 2-8°C. Do not freeze. Product is shipped at room temperature.
Note	For research use only

### Applications

The chemiluminescent substrate solution is used to trigger HRP labels to give a quite rapid light emission in chemiluminescence technology.

### Attention (Important Product Information)

1. The chemiluminescent substrate solution is highly sensitive. Optimization of the antigen, antibody and HRP conjugate concentrations may be required.
2. To decrease background signal, choosing a suitable blocking buffer is very important. Based on your plates and materials, the reasonable experimental conditions should be figure out.
3. To limit nonspecific signal due to unsuitable reagent solutions, please choose an appropriate assay buffer solution for the experiment.
4. To reduce cross-contamination between positive samples and negative samples, please add samples in the correct way and sequence.
5. Do not use azide as a preservative because azide is a known inhibitor of HRP.
6. Measure the relative light units (RLU, ~425nm) in a luminometer within 5 minutes, because over time, the signal will gradually decrease.
7. If the signal value is not available, check whether the buffer and reagent are expired. Do not use an expired buffer and reagent. The components of different batch should not be mixed used because it may lead to

---

incorrect results.

### Procedure for assay

1. Prepare materials and tools for your experiment, such as plate, protein or antibodies, coating buffer, wash buffer, blocking buffer, dilution buffer and so on.
2. Coat the plate with target proteins or antibodies, wash and block the plate, add samples and HRP conjugates according to correct experimental procedures.
3. Prepare the triggering Working Solution, mix equal parts of the Solution A (Luminol-Based Solution) and Solution B (Peroxide Solution). This Working Solution is stable for approximately 8 hours at room temperature.

*Note: Exposure to the sun or any other intense light can harm the Working Solution. For best results keep the Working Solution in an amber bottle and avoid prolonged exposure to any intense light Short-term exposure to typical laboratory lighting will not harm the Working Solution.*

4. Add an appropriate volume of Working Solution to each well, such as add 100 $\mu$ L to each test. Because the signal from the chemiluminescence substrate ramps up very quickly and optimal signal production lasts only for 5-10 min, the time of adding the chemiluminescence substrate to the wells is very important, we recommend using a multichannel pipette to add the substrate to the wells as quickly as possible, mix liquid in wells for 1 minute using a microplate mixer, and then read the signal produced. Typically, reading each well for 0.1-1 sec will provide good signal with this substrate.
5. Measure the relative light units (RLU,  $\sim$ 425nm) in a luminometer equipment between 1-5 minutes after adding the substrate. Longer periods between adding the substrate and evaluating the plate may result in decreased signal intensity. Due to equipment differences, the final read value of relative light units (RLU) may be different.

*Note: The peak emission wavelength is given for reference; however, for best sensitivity, measure total light output using a luminometer. Signal also can be measured in a test-tube luminometer. For test-tube applications, increase the Working Solution volume as needed.*

### Shipping and Storage

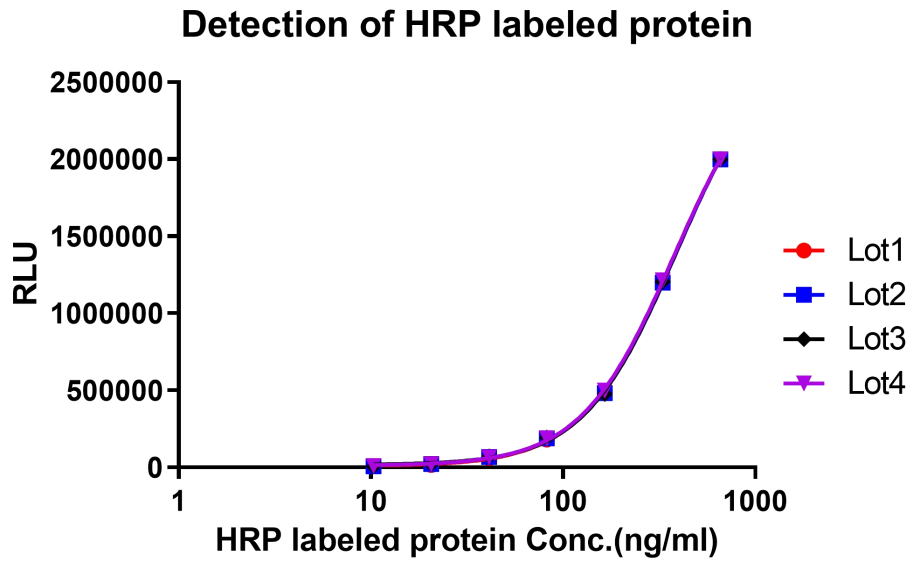
The product is stable for up to 1 year from the date of manufacture at 2-8°C. Do not freeze.

The product is shipped at room temperature. Upon receipt, please store the buffer at room temperature away from light. Do not use reagents past their expiration date.

Before use, equilibrate the buffer to room temperature.

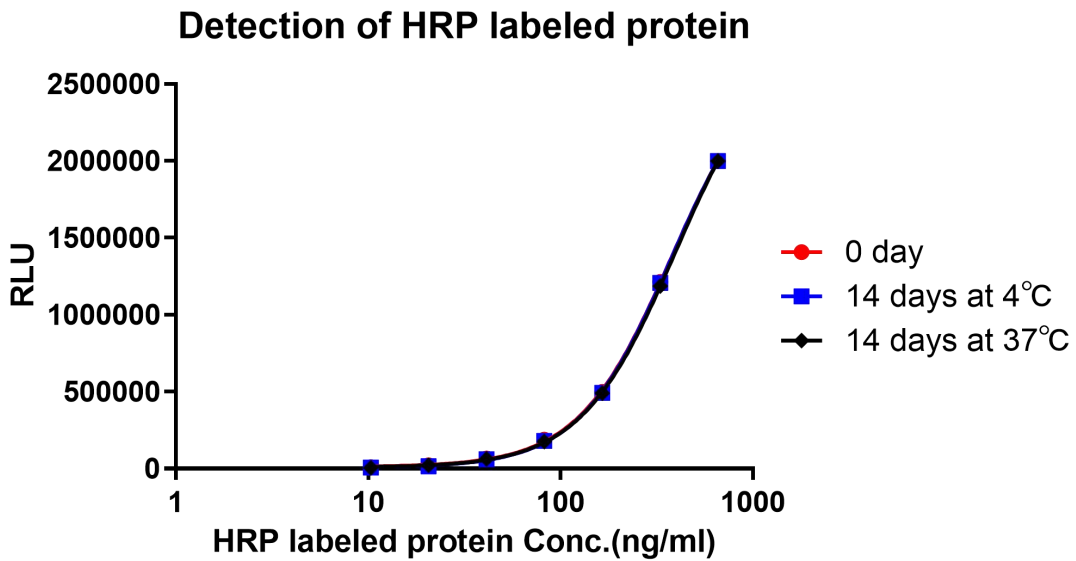
## Figures

### Batch consistency of ABK-002



The Product ABK-002 is high batch-to-batch consistency.

### Stability of ABK-002



The Product ABK-002 is high stability. The accelerated stability of the Chemiluminescent Substrate (ABK-002) within 14 days at 37°C with no more than 5% performance decrease.