

	Product Specification
Product :	<b>Bio-Doc ECL Reagent</b>
Product Number :	<b>BDWE100 / BDWE250</b>
Package :	Reagent A 100 mL + Reagent B 100 mL Reagent A 250 mL + Reagent B 250 mL
Storage :	2-8 °C

## Description

The principle of BioDoc ECL Reagent is based on chemiluminescence and is very convenient to detect the Horseradish peroxidase (HRP) activity in many assays such as Western blotting <sup>,</sup> Southern and Northern. HRP catalyze the chemiluminescent oxidation of cyclic diacylhydrazides such as luminol by hydrogen peroxide ( $H_2O_2$ ). BioDoc ECL Reagent can enhance the luminol-dependent chemiluminescence and can be wildly use to detect the present of HRP-conjugated antibodies or streptavidin which binding to antigen or nucleotide sequence respectively. BioDoc ECL Reagent is highly compatible with any other HRP based detection methods, such as Western blot, dot plot, or protein array, whether in PVDF, Nylon or Nitrocellulose membranses in most commonly used buffer solutions.

## The Detection of Proteins Protocol

1	Mix the solution A 1 ÷ 1 with solution B in BioDoc ECL Reagent and incubate the mixture for 1 min in room temperature.
2	Add the sufficient mixture solution to cover the membrane $(0.1 \text{ mL/cm}_2)$ . It is not needed incubation time.

- 3 Discard the excess mixture in membrane and wrap the membrane in saran wrap. Carefully and gently remove the air bubbles from the membrane.
- 4 Cover the membrane in plastic wrap and expose to X-Ray film, or cool CCD image system for an appropriate duration.

Place the membrane in the film cassette and keep the protein side up. Turn off the lights and use safety light. Then place a sheet of film on the membrane and close the cassette and expose for 10-90 seconds (The actual exposure time is adjusted according to the experiment).

- 5 Open cassette and transfer the exposed film to developing machine. Then place a new film on the membrane and expose again.
- 6 The exposure time of second film can be adjusted by the intensity of first film.
- 7 If the intensity was too high, please wait up to 10 minutes before re-exposing.

## Note

1	Keep the membrane in wet after first antibody incubation.
2	Keep the BioDoc ECL Reagent fresh and do not reuse the BioDoc ECL Reagent.
3	Please use new tip to avoid contamination.
4	Step 1-3 shown in procedural cannot be handled in darkroom.
5	The antibody on the membrane can be remove by using BioDoc Western Blot Stripping Buffer (BDSB01) and

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reprobe again.