Product Profile

Product Name:	*EZ-ECL Kit*
Product Catalog Number	*20-500-100*
Unit Size Availability:	See Kit Components Below(p.1)
Formulation:	Kit
Specified Storage Conditions:	2-8°C
Stability: (Under Specified Handling & Storage)	*Please Refer to the Product Label*

Important Note! Please read the *MSDS* and *Product Profile* carefully in their entirety *before* using this material for possible safety precautions and potential hazards.

Product Description

EZ-ECL Kit is a complete kit with ready-to-use reagents designed for the detection of immobilized proteins(Western Blotting) or immobililized nucleic acids(Southern or Northern), conjugated with Horseradish Peroxidase (HRP) directly or indirectly. The use of enhanced chemiluminescence was introduced by Thorpe and Kricka.[1, 2] In the presence of hydrogen peroxide (H_2O_2), Horseradish Peroxidase (HRP) catalyzes the oxidation of cyclic diacylhydrazides, such as luminol. Immediately following the oxidation, the luminol, in an excited state (i.e. the intermediate reaction product) decays to the ground state by emitting light. Strong enhancement of the light emission is produced by enhancers such as phenolic compounds. Using this method, it is possible to detect membrane immobilized specific antigens or sequences of nucleic acids which are labeled directly with HRP or indirectly with HRP-labeled antibodies/streptavidin.

Kit Reagents

Catalog Number	*20-500-120A*	*20-500-120B*
Name	Luminol and Enhancer	Stable Peroxide Solution
Contents	EZ-ECL Solution A	EZ-ECL Solution B
Volume	60ml	60ml
Storage Conditions	2-8°C	2-8°C

Precautions

The EZ-ECL Kit Contains Potentially Hazardous Components. Please Refer To The MSDS Before Handling This Product For Proper Precautions And Personal Protection Equipment.

Blotting

Blotting in a general sense, is used to refer to the transfer of protein, RNA or DNA molecules from a relatively thick acrylamide or agarose gel to a paper-like membrane(usually nylon or nitrocellulose) by capillarity or an electric field, while preserving the spatial arrangement. Once on the membrane, the molecules are immobilized, typically by baking or by ultraviolet irradiation, and can then be detected at high sensitivity by hybridization(i.e. in the case of DNA or RNA), or antibody labeling(i.e. in the case of protein). RNA blots are known as *Northern* Blots, DNA blots are known as *Southern* and *Western* Blots are Protein blots. In *Northwestern* Blotting, protein is transferred but is probed with specific RNA.

Western Blotting

Western Blotting is an electro-blotting method in which proteins are transferred from a gel to a thin, rigid support (i.e. most often nitrocellulose) and detected by binding of labeled antibody.

Chemiluminescence

Chemiluminescence is a process by which light is emitted as a reaction proceeds. In nature, the bioluminescent display or flashes of fireflies (i.e. actually lampyrid beetles) are species-specific signals which serve as behavioral mechanisms of reproductive isolation. It is termed bioluminescence when it occurs in a living organism. The Chemiluminesence process is increasingly utilized not only to assay ATP (i.e. using firefly Luciferase as an ATP-ase) which is used routinely as a sensitive ATP assay system, but also used in the production of toxic oxygen species by activated phagocytes using either luminol or lucigenin as bystander substrates that release light when oxidized. Leukocytes (e.g. neutrophils) are known to emit small amounts of light when their oxidative metabolism is stimulated.

Luciferase is a generic term for a class of oxidative enzymes used in bioluminescence which is also shared by many other organisms, mostly marine or sea-living organisms. In insects, specialized abdominal cells contain the chemical luciferin which combines with oxygen (i.e. from the abdominal trachea) to form an inactive molecule oxyluciferin. When combined with ATP, the reaction proceeds emitting light, thus releasing the oxyluciferin and AMP from the enzyme's surface. The enzyme, Luciferase from firefly abdomen catalyses the production of light in the reaction between luciferin and ATP. The male firefly uses luciferase to attract females of the same species; the males recognizing conspecific females by their flash response and the females recognizing conspecific males by their flash pattern. This series of reciprocal responses provides a so-called "continuous check" on the species identity of potential mates. Luciferase is utilized in the laboratory in chemiluminescence bioassays for ATP. The gene for the Luciferase enzyme has been isolated, placed in the genes of other organisms and utilized to follow the synthesis and/or the expression of other genes (i.e. as a reporter gene).

Some Predominant Characteristics of the EZ-ECL Test Kit include:

- § High Sensitivity, non-radioactive detection system
- § Stable hard-copy results on film
- § Only Small Amounts of antibody are required
- § Detection may be achieved with short exposure times(minutes)
- § High Resolution
- § Easy to Understand Detailed Protocols
- § Protocol for Western Blotting and Chemiluminescence detection
- § Protocol for Southern/Northern Blotting & Chemiluminescence Detection
- § Performance-Tested with Good Yields

Storage & Handling Precautions and Disclaimer

For *In Vitro* Use Only.

The product should be stored under specified conditions of 2-8°C. The product should not be left in the light for prolonged periods. When stored in the dark under specified conditions, the product is stable until the expiry date.

Quality Control

Test	*Specification*
Osmolality of Solution A:	150-250 mOsm/kg
Osmolality of Solution B:	240-280 mOsm/kg
Performance Test: Dot-Blot Detection	Pass Test
pH of Solution A	9.0-10.0
pH of Solution B	8.0-9.0
Sterility:	Sterile

Auxiliary Products

Product Name	Catalog Number	Storage Temperature
DEPC-Treated Water	01-852-1	15-30°C
Water-Saturated Phenol	01-860-1	2-8°C
UltraPure Water	01-866-1	15-30°C
TAE Buffer 50X Buffer	01-870-1	Room Temperature(15-30°)
TBE Electrophoresis Buffer Concentrate(5X)	01-871-1	Room Temperature(15-30°)
Acrylamide/bis-Acrylamide (19:1) (T=40%,C=3.3%)Solution	01-872-1	2-8°C
Acrylamide/bis-Acrylamide (29:1) (T=40%,C=3.3%)Solution	01-874-1	2-8°C
Acrylamide/bis-Acrylamide (37.5) (T=40%, C=2.6%) Solution	01-876-1	2-8°C
RBC Lysis Solution	01-888-1	2-8°C
EZ-Hybridization Solution	01-889-1	2-8°C
SDS Solution (10%)	01-890-1	Room Temperature(15-30°)
RNA Save	01-891-1	Room Temperature(15-30°)
Quick Load 5X Conc. (PCR Loading Solution)	01-892-1	2-8°C
EZ-Plant	01-893-1	2-8°C
RNase-Exitus Plus™	01-897-1	Room Temperature(15-30°)
DNA- Exitus Plus™	01-898-1	Room Temperature(15-30°)
Random Primer DNA Labeling Kit	20-101-25	-20°C
DNA Isolation Kit for 150-300 Isolations	20-200-300	2-8°C
EZ-RNA Total Isolation Kit	20-400-100	2-8°C
EZ-RNA II Total RNA Isolation Kit without Chloroform, with BCP	20-410-100	2-8°C
EZ-DNA Genomic DNA Isolation Kit	20-600-50	Room Temperature(15-30°)
EZ-First Strand cDNA Isolation Kit	20-800-50	-20°C
Note: For a list of Antibiotics, Serum, Reagents and other Supplements, please refer to our Product Catalog, Product Guides, Product Profiles and Internet Site.		

References:

1) Chomczynski, P. and Sacchi, N. *Analytical Biochemistry*, 162:156-159(1987)
2) Biological Industries (BI), Product Insert, "EZ-ECL Chemiluminescence Detection Kit for HRP Kibbutz Beit HaEmek.
3) Sullivan Jr. John B. Krieger, Gary R. Hazardous Materials Toxicology: Clinical Principles of Environmental Health. Williams & Wilkins: Baltimore, Maryland, pps.732-747; 1093-1096, 1992.
4) Barile, Frank A. Clinical Toxicology: Principles and Mechanisms. CRC Press: Boca Raton, Florida, 2004.
5). Thorpe, G.H.G. and Kricka, L.J., *Methods in Enzymology*, 133,331-353(1986)
6). Thorpe, G.H.G. and Kricka, L.J., Moseley, S. B. and Whitehead, T.P., *Clin. Chem.*, 31(8) :1335-1341(1985)
7). Riko, I., *et. al., Analytical Biochemistry*, 231: 170-174(1995)

Biological Industries