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You can find your partners, iNtRON Distributor in WebPage.

- **No mix Just Spray**
- **Fast Detection**
- **High Sensitivity and Stronger Signal**
- **High Contrast Signals**
- **Steady Signal Duration Time**

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Sangdaewon-dong, Joongwon-gu,
Sungnam-si, Kyeonggi-do, Korea
iNtRON Biotechnology, Inc.

Instruction Manual

WEST-one™

Western Blot Detection System

REF	16034
RUO	100 ml
8℃ / 2℃	

Sensitive and Stronger HRP Substrate
for Chemiluminescent Western Blot Assay

iNtRON Biotechnology

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TABLE OF CONTENTS

Kit Information	Description	2
	Characteristics	2
	Kit Contents	3
	Storage	3
	Applications	3
	Important Notes Before Starting	4
	Additional Required Equipment	5
	Quality Control	6
	Technical Assistance	6
	Product Use Limitations	6
Protocols	Quick Guide	7
	Protocol	8
Additional Information	Troubleshooting Guide	12
	Technical Advise	15
	Experimental Information	17
	Relative Products	20
	Global Distributors	21

DESCRIPTION

The WEST-one™ Western Blot Detection System is a non-radioactive method for detection of immobilized specific antigens thorough horse radish peroxidase (HRP)-labeled antibodies. The WEST-one™ Western Blot Detection System is quite unique system which is combined consist of substrate solution and enhancer solution maintaining its stability. (No Require to mix the substrate and enhancer solution. Just spray on to membrane) The WEST-one™ Western Blot Detection System utilizes a advanced version of the chemiluminescent HRP substrate luminol that results in the fast and more sensitive detection of an antigen while providing a long lasting signal and the strong signal to noise ratio of any competitor's

CHRACTERISTICS

- **Just Spray** : Spray the WEST-one™ Kit on the membrane. It has very simple step, which consist of reaction detection solution and membrane blot and exposure to X-ray film.
- **Fast** : Specific protein detection may be achieved in less than 1-10 minute.
- **High sensitivity** : WEST-one™ Kit is able to detect picograms level of antigen on membrane blot.
- **Steady** : Signal duration time of WEST-one™ Kit is 7-15 times longer than that of other company's products.

KIT CONTENTS

Component	Amount
WEST-one™ Detection Solution	100 ml
Instruction Manual (Hand book)	1 ea

STORAGE

- Storage : On receipt all components should be stored at 2 - 8℃. The WEST-one™ Western Blot Detection System is sensitive to light. Long term storage of the individual reagent should be in the light blocked containers in which they are provided.
- Expiry : The components of WEST-one™ Western Blot Detection System are stable for at least 12 months when stored under the recommended conditions

APPLICATIONS

- Western Blotting using chemiluminescence (HRP detection)

IMPORTANT NOTES BEFORE STARTING

- For best results, it is important to optimize all system components including sample amount, antibody concentrations and the membrane and blocking reagents.
- The required antibody concentrations are more dilute than those used with colorimetric HRP detection. In order to optimize the appropriate concentrations, perform a systematic dot blot analysis.
- Using the optimized blocking buffer can increase sensitivity and prevent nonspecific signal caused by cross-reactivity between the antibody and the blocking reagent.
- Avoid using milk as a blocking reagent when using avidin/biotin systems because milk contains endogenous biotin.
- Use a sufficient volume of wash buffer, blocking buffer, and antibody solution to cover blotted membrane and ensure that it never becomes dry. Large volumes of blocking and wash buffer may reduce background.
- All equipment (especially metalic device - scissors, forceps) must be clean and free of contaminants.
- To reduce non-specific signal, add Tween-20 (final concentration of 0.05%) to the blocking buffer and when preparing all antibody dilutions.
- Do not use sodium azide as a preservative for buffers, sodium azide works an inhibitor of HRP.

ADDITIONAL REQUIRED EQUIPMENT

- **Completed Western blot membrane** : Nitrocellulose membrane or PVDF membranes (0.22μm pore size).
- **Dilution Buffer** : Tris Buffered Saline (TBS) or Phosphate Buffered Saline (PBS).
  TBS, pH 7.6 : 8 g NaCl, 20 ml 1 M Tris-HCl, pH 7.6, Dilute to 1000 ml with distilled water then check pH
  PBS, pH 7.5 : 11.5 g Na_2HPO_4 Anhydrous (80 mM), 2.96 g $NaH_2PO_4.2H_2O$ (20 mM), 5.84 g NaCl (100 mM). Dilute to 1000 ml with distilled water then check pH.
- **Wash Buffer** : TBS-T or PBS-T (TBS or PBS + 0.05% Tween 20)
- **Blocking Reagent** : 5% non-fat dried milk in TBS-T or PBS-T (Gelatin, Casein and Bovine Serum Albumin (BSA) may also be used as alternative blocking reagents)
- **Primary antibody** : Extremely variable, from 1:10 ~ 1:100,000. The optimal dilution to use depends on the specific primary antibody and the amount of antigen on the membrane.
- **HRP-conjugated secondary antibody** : commonly working dilution at 10-50ng/ml (i.e., 1:20,000-1:100,000 from a 1mg/ml stock). The optimal dilution to use varies depending on the specific conjugate and the amount of antigen on the membrane.
- **Film, film cassette, developing and fixing reagents** : For processing autoradiographic film (X-ray film).
- **Orbital shaker** : For agitation of membrane during incubations.

QUALITY CONTROL

Every batch of the product is functionally tested in a Western blotting application to ensure minimal lot to lot variation.

TECHNICAL ASSISTANCE

At iNtRON we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of iNtRON products. iNtRON customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at iNtRON. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques. For technical assistance and more information please call iNtRON Technical Service Department or local distributors.

PRODUCT USE LIMITATIONS

WEST-one™ Western Blot Detection System is developed, designed, and sold for research purpose only. They are not to be used for human or animal diagnosis of diseases. Do not use internally or externally in humans or animals. Be careful in the handling of the products.

QUICK GUIDE

PROTOCOL

A. Electrophoresis and blotting

1. **Perform electrophoresis and blotting according to usual techniques. Proteins should be transferred to PVDF or Nitrocellulose membrane. Blots may be used immediately or stored in a dry condition at 2 – 8°C for up to 3 months.**
   Note 1 : PVDF should be pre-wetted in 100% methanol, washed in distilled water for 5 ~ 10 min. and equilibrated in transfer buffer for at least 10 minutes before blotting.

B. Block the membrane

1. **Block non-specific binding sites by immersing the membrane in 5% non-fat dried milk, 0.05% (v/v) Tween 20 in PBS or TBS (PBS-T or TBS-T) for 1 hour at room temperature (RT) on an orbital shaker. Alternatively, membranes may be left in the blocking solution overnight in a refrigerator at 2 – 8℃.**
   Note : The combination of non-fat dried milk and Tween 20 should be sufficient for most applications. Optimum Tween 20 concentrations will vary to suit specific experiments, but a 0.05% Tween 20 concentration is suitable for most blotting applications.
2. **Briefly rinse the membrane with 3 times of Wash Buffer**

C. Primary antibody treatment

1. **Pour off Wash Buffer, dilute the primary antibody in Wash Buffer. The dilution factor should be determined empirically for each antibody.**
2. **Incubate the membrane in primary antibody solution for 1 hour at room temperature on an orbital shaker.**
   Note : Incubation times and temperatures may vary and should be optimized for each antibody. The conditions indicated are recommended starting points.
3. **Pour off primary antibody solution from membrane, then wash the membrane for 10 min. at RT 3 times with Wash Buffer**

D. Secondary antibody Treatment

1. **Pour off Wash Buffer, dilute the secondary antibody in Wash Buffer. The dilution factor should be determined empirically for each antibody.**
2. **Incubate the membrane in secondary antibody solution for 1 hour at room temperature on an orbital shaker.**
   Note : Incubation times and temperatures may vary and should be optimized for each antibody. The conditions indicated are recommended starting points.
3. **Pour off secondary antibody solution from membrane, then wash the membrane for 10 min. at RT 3 times with Wash Buffer**

E. Detection

1. **Spray the WEST-one™ Detection Solution to give sufficient to cover the membranes.**
   Note : The volume of one click is 500 µl. The final volume of detection reagent required is 0.1 ml/cm^2
2. **Incubation for 1 minute at RT without agitation.**
   Note : The reaction for 1 minute is sufficient for detection of specific protein.
3. **Drain off excess detection reagent by holding the membrane vertically and touching the edge of the membrane with tissue paper.**
   Note : For less background, excess detection reagent on membrane have to be removed.
4. **Wrap membranes in transparent thin plastic wrap and gently smooth out air bubble. And then place the blots, protein side up, in the film cassette.**
   Note : It is necessary to work quickly once the membranes have been exposed to the detection system.
5. **Switch off the lights and carefully place a sheet of autoradiographic film on top of the membranes, close the cassette and expose for dozens of minutes.**
   Note : Do this step in a dark room using red safe light. How long to continue the exposure depend on the amount of target protein on the membrane. Exposure from 30 seconds to 5 minutes is sufficient to detection of abundant protein.

(*continued*)

Note : Film must remain dry during exposure. For optimal results, Make sure excess substrate is removed from the membrane and the membrane protector.
Note : Use gloves during the entire film-handling process.
Note : Never place a blot on developed film, as there may be chemicals on the film that will reduce signal

6. Develop film using appropriate developing and fixing reagent

TROUBLESHOOTING GUIDE

Problem	Causes	Comments
Weak or no signal	Too much HRP in the system depleted the substrate and caused the signal to fade quickly	Dilute HRP-conjugate at least 10-fold
	Insufficient quantities of antigen or antibody	Increase amount of antibody or antigen
	Inefficient protein transfer	Optimize transfer condition
	Reduction of HRP or substrate activity	To test the activity of the HRP detection system in the darkroom , prepare 1-2 mL of the WEST-one™Western Blot Detection System in a clear test tube. With the lights turned off, add 1 µL undiluted HRP-conjugate to the Working Solution. The solution should immediately emit a blue light that will fade over the next several minutes.

TROUBLESHOOTING GUIDE

Problem	Causes	Comments
High background	Inadequate blocking	Optimize blocking conditions
	Too much HRP in the system	Dilute HRP-conjugate at least 10-fold
	Inappropriate blocking reagent	Try a different blocking reagent
	Inadequate washing	Increase length, number or volume of washes
	Film has been overexposed	Decrease exposure time
	Antigen or antibody is too concentrated	Dilute antigen or antibody working solution
Reverse image on film	Too much HRP in the system	Dilute HRP-conjugate at least 10-fold
Membrane has brown or yellow Bands		
Signal duration is too short (less than few hours)		

TROUBLESHOOTING GUIDE

Problem	Causes	Comments
Spots within the protein bands	Unevenly hydrated membrane	Perform manufacturer's recommendations for hydrating membrane properly
	Bubble between the film and the membrane	Remove all bubbles before exposing blot to film
Speckled background on Film	Aggregate formation in the HRP-conjugate	Filter conjugate through a 0.2 μm filter or centrifuge and use supernatant
Nonspecific bands	Inadequate blocking	Optimize blocking conditions
	Too much HRP in the system	Dilute HRP-conjugate at least 10-fold

TECHNICAL ADVICE

◈ Critical parameters

1. WEST-one™ Western Blot Detection System can be used with both nitrocellulose and PVDF membranes. However the improvement observed is likely to be more significant with PVDF membranes than with nitrocellulose. In addition the prolonged light output is a feature of WEST-one™ Western Blot Detection System with PVDF membranes. Therefore in order to achieve the best results with WEST-one™ Western Blot Detection System the use of PVDF membranes is recommended.
2. During immunodetection, sufficient solution should be used to adequately cover the membrane. Containers should be agitated gently on a mixer platform.
3. While non-fat dried milk is strongly recommended as the membrane blocking agent, Gelatin, Casein and Bovine Serum Albumin (BSA) may also be used as alternative blocking reagents with the WEST-one™ Western Blot Detection System.
4. When washing, the volume of wash buffer should be as large as possible ; 4 ml of buffer per cm^2 of membrane is suggested. Brief rinses of the membrane in wash buffer before incubating will improve washing efficiency.
5. It is advisable to avoid the use of containers that are polystyrene based to mix WEST-one™ Western Blot Detection System as the solution will turn milky and produce a precipitate. Other types of containers, such as polypropylene, poly-ethylene, polymethyl-pentene and glass are all suitable for use.
6. If exposure times of less than 5 seconds are routinely required, it is recommended that the antibodies used are further diluted as it is difficult to perform such short exposures.

TECHNICAL ADVICE

◈ Stripping and reprobing membranes

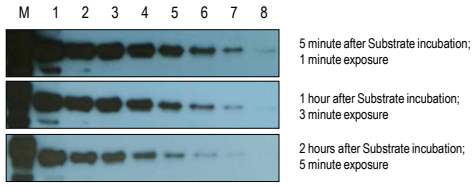
The complete removal of primary and secondary antibodies from the membrane is possible following the protocol outlined below.
The membranes may be stripped of bound antibodies and reprobed several times. Membranes should be stored wet wrapped in plastic wrap in a refrigerator (2–8 ℃) after each immunodetection.

1. Submerge the membrane in stripping buffer (100 mM 2-Mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl pH 6.7) and incubate at 50℃ for 30 minutes with agitation.
2. Wash the membrane 2 times for 10 minutes in large amount of Wash Buffer at room temperature.
3. Block the membrane in 5% non-fat dried milk in Wash Buffer for 1 hour at room temperature.
4. Repeat the immunodetection protocol.

EXPERIMENTAL INFORMATION

◈ Prolonged Duration Time

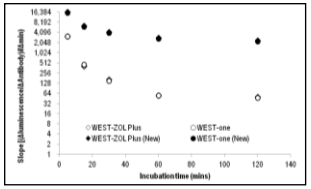
Antibody was diluted with dilution buffer in ½ serial dilution. And then spray the WEST-one™ Western Blot Detection System on the membrane. The other step progress the normal western blotting method.



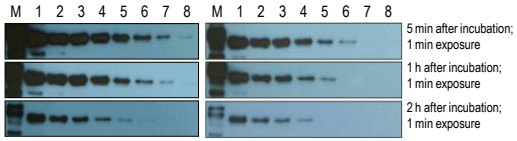
Lane M; ProView Western Protein Marker, **lane 1**; 250 pg of antigen, **lane 2**; 125 pg of antigen, **lane 3**; 62.5 pg of antigen, **lane 4**; 31.3 pg of antigen, **lane 5**; 15.6 pg of antigen, **lane 6**; 7.8 pg of antigen, **lane 7**; 3.9 pg of antigen, **lane 8**; 2.0 pg of antigen.

EXPERIMENTAL INFORMATION

◈ Enhanced Sensitivity



Chemiluminescence detection with luminometer
The light signal of WEST-one™ Western Blot Detection System is more sensitive and stable than our old version of product.



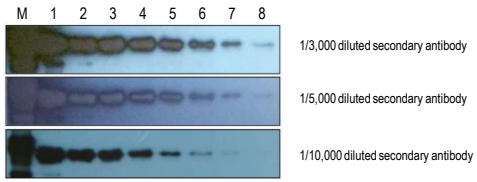
[WEST-one™ Kit] **[Our old version product]**

Lane M; ProView Western Protein Marker, **lane 1**; 250 pg of antigen, **lane 2**; 125 pg of antigen, **lane 3**; 62.5 pg of antigen, **lane 4**; 31.3 pg of antigen, **lane 5**; 15.6 pg of antigen, **lane 6**; 7.8 pg of antigen, **lane 7**; 3.9 pg of antigen, **lane 8**; 2.0 pg of antigen.

EXPERIMENTAL INFORMATION

◈ Save antibody, effective detection

WEST-one™ Western Blot Detection System can be detect the target gene under various concentration of secondary antibody. The Kit produced the effective detection sensitivity with less amount of secondary antibody.



Lane M; ProView Western Protein Marker, **lane 1**; 250 pg of antigen, **lane 2**; 125 pg of antigen, **lane 3**; 62.5 pg of antigen, **lane 4**; 31.3 pg of antigen, **lane 5**; 15.6 pg of antigen, **lane 6**; 7.8 pg of antigen, **lane 7**; 3.9 pg of antigen, **lane 8**; 2.0 pg of antigen.

RELATIVE PRODUCTS

Product Name	Cat. No
PRO-PREP™ Protein Extraction Solution	17081
SMART™ BCA Protein Assay Kit	21071
WEST-ZOL® plus Western Blot Detection System	16024
ProView™ Western Protein Marker	24086
Blocking One-P	ITN-05999-84
PRO-MEASURE™ Protein Measurement Solution	21011

GLOBAL DISTRIBUTORS

◈ Molecular Reagent (MR)

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◈ Mol. Diagnosis (MD)

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◈ MR and MD

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