Cat. # T7122A

For Research Use

TaKaRa

Western BLoT Rapid Detect v2.0

Product Manual

Note: Store all components at -20℃

v201902

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I. Description

Western BLoT Rapid Detect v2.0 is a Western blot detection reagent kit used to detect primary antibodies using an HRP-labeled IgG Detector instead of a labeled secondary antibody.
IgG Detector, the main component of this product, is a protein particle with approximately 100 molecules of the protein that bind to the Fc region of an antibody on surface of the particle, and is labeled with about 50 molecules of HRP. This reagent enables quick, high-sensitivity detection and can be used for simultaneous detection of multiple primary antibodies. Moreover, as 10X Dilution Buffer v2 contains a blocking component, this product allows for higher sensitivity and simpler use.

For the highest sensitivity and quickest detection for Western blotting, use this product in combination with the Western BLoT HRP Substrate series.

II. Components

1.	IgG Detector Solution v2 (HRP labeled)	250 µl
2.	10X Dilution Buffer v2	60 ml
3.	Enhancer for Mouse IgG v2	250 µl
4.	Marker Detection Reagent v2	50 µl

III. Storage

-20℃

Note : Store IgG Detector Solution v2 (HRP labeled) at -20℃ until immediately prior to use; store at -20℃ immediately after use. Do not store at 4℃ or room temperature.

IV. Materials Required but not Provided

- Blocking solution
  * Protein blocking solutions (1% BSA in TBS-T, etc.) will result in higher signals than nonfat milk. If you use nonfat milk, use reagent grade.
- TBS-T: Tris Buffered Saline with Tween 20 (TBS-T) Tablets, pH 7.6 (Cat. #T9142)
  (TBS-T composition: 150 mM NaCl, 10 mM Tris-HCl, 0.1% Tween 20, pH 7.6)
- Prepare all other solutions and equipment required for Western blotting.
  It is recommended to use chemiluminescence for detection.

V. Precautions for Use

The following are important points when using this product. Read before use.

1. This product can detect most primary antibodies. However, detection for the primary antibody of mouse IgG_1, goat IgG, and human IgG_3 is harder due to the low binding ability of the IgG Detector. When mouse IgG_1 is used as primary antibody, add the Enhancer for Mouse IgG v2 to the IgG Detector Solution v2 to detect well the primary antibody. When goat IgG or human IgG_3 is used as primary antibody, this product can be used in only the case of the Secondary Antibody Sensitization Protocol (Section VI-2c).
2. Since IgG Detector consists of an Fc-region binding protein, it cannot be used for fragmented antibodies such as $F(ab')_2$ and Fab, etc. In the use of a mouse antibody as a primary antibody, it is possible to use the IgG Detector by adding Enhancer for Mouse IgG v2. And, in the Secondary Antibody Sensitization Protocol (Section VI-2c) and the Reprobe Sensitization Protocol (Section VI-2d), detection is possible when anti-L chain and anti-H+L chain antibodies are used as the secondary antibodies.
3. Because higher signals can be obtained compared to conventional Western blotting methods that utilize labeled secondary antibodies, the primary antibody can be used at a lower concentration. However, preliminary experiments are necessary since the increase in sensitivity depends on the properties of the antigen and antibody.
4. In the Rapid Protocol (Section VI-2a), the primary antibody and IgG Detector are mixed beforehand. If the primary antibody solution contains sodium azide as a preservative, adjust the final concentration of sodium azide to no more than 0.001%, as higher concentrations may inhibit HRP activity.
5. Simultaneous detection of multiple antibodies is possible by simply adding multiple primary antibodies into a primary antibody reaction solution. Because the IgG Detector reactivity differs depending on the antibody, optimization of dilution ratios is required for each antibody beforehand.
6. Nitrocellulose and PVDF membranes can be available. A pore size of 0.20 μm is recommended for PVDF membranes; using a membrane with a pore size of 0.45 μm may increase background.

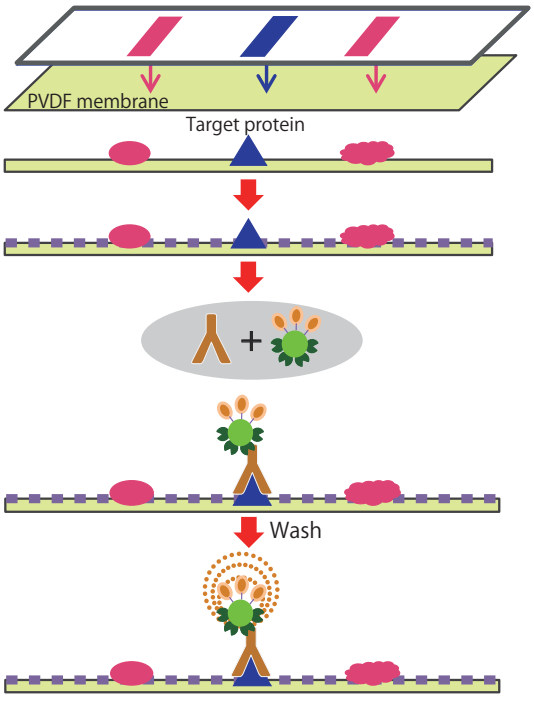
VI. Protocol

This product is suitable for various applications. Four protocols are described below.

Protocol	Explanation
VI-2a Rapid Protocol	• Perform a one-step quick detection by mixing primary antibody and IgG Detector (Time required: 1 hour). • For high-sensitivity detection, the time for blocking and incubation with antibodies is extended (Time required: 2 hours). • A labeled secondary antibody is not required. • Simultaneous detection with multiple primary antibodies (immunized animal species can be different) is possible.
VI-2b 2 Step High Sensitivity Protocol	• Perform a two-step reaction, which is the same as the standard method, using IgG Detector instead of a labeled secondary antibody. It can provide higher sensitivity detection than the standard method. A labeled secondary antibody is not required. • Simultaneous detection with multiple primary antibodies (immunized animal species can be different) is possible.
VI-2c Secondary Antibody Sensitization Protocol	• After performing the standard method with a labeled secondary antibody, sensitivity is further increased by additional use of IgG Detector (three-step reaction). • Detection cannot be performed with the V-2a or V-2b protocols when the primary antibody type is Goat IgG or Human IgG_3, but these antibodies can be used in this protocol.
VI-2d Reprobe Sensitization Protocol	• Re-detect signals using IgG Detector on a membrane that previously yielded weak signal using a labeled secondary antibody. A very small amount of antigen may be detected, even when it cannot be detected with the standard methods.

[Protocol overview] Procedures until the blocking step are the same in VI-2a to VI-2c.

VI-2a. Rapid Protocol



PVDF membrane with transferred protein.

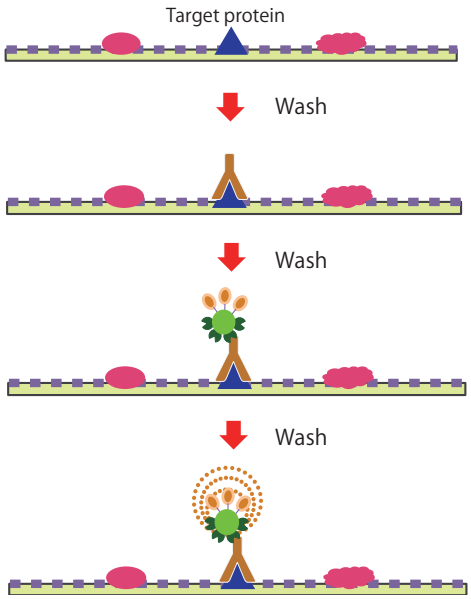
Block with blocking buffer for 5 min at room temperature.

Incubate in a solution*3, in which primary antibody*2 and IgG Detector are diluted with IgG Detector Dilution Buffer*1, at room temperature for 30 min (IgG Detector is diluted 2,000-fold).

Wash with TBS-T (5 min x 5).

Detect with Western BLoT HRP Substrate series.

VI-2b. 2 Step High Sensitivity Protocol



Block in blocking solution at room temperature for 1 hour.
Wash with TBS-T (5 min x 3).

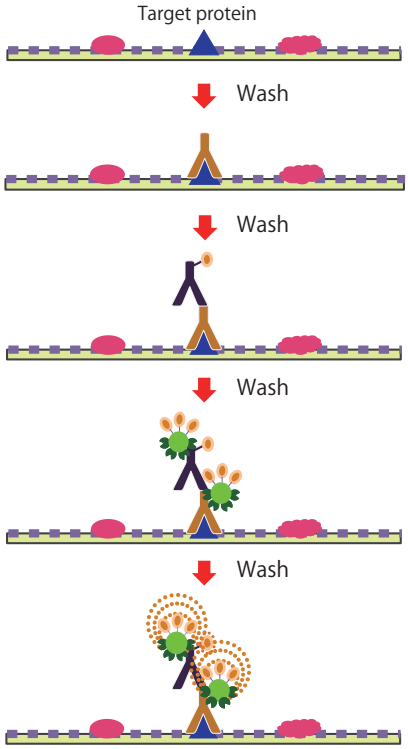
Incubate with primary antibody*2 (dilute with primary antibody dilution buffer)*3 at room temperature for 1 hour.
Wash with TBS-T (5 min x 3).

Incubate with IgG Detector (1,000 - 4,000-fold dilution with IgG Detector Dilution Buffer*1) at room temperature for 1 hour.

Wash with TBS-T (5 min x 5).

Detect with Western BLoT HRP Substrate series.

VI-2c. Secondary Antibody Sensitization Protocol



Block in blocking solution at room temperature for 1 hour.
Wash with TBS-T (5 min x 3).

Incubate with primary antibody (dilute with primary antibody dilution buffer) at room temperature for 1 hour.
Wash with TBS-T (5 min x 3).

Incubate with secondary antibody (dilute with primary antibody dilution buffer).

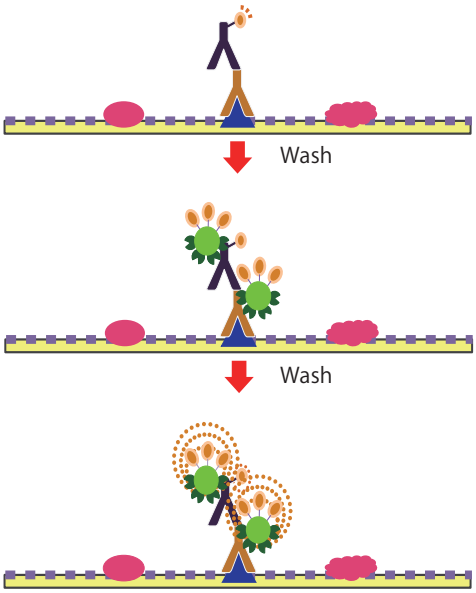
Wash with TBS-T (5 min x 3).

Incubate with IgG Detector (1,000 - 4,000-fold dilution with IgG Detector Dilution Buffer*1) at room temperature for 1 hour.

Wash with TBS-T (5 min x 5).

Detect with Western BLoT HRP Substrate series.

VI-2d. Reprobe Sensitization Protocol



Pre-used PVDF membrane (Immerse in TBS-T; do not allow to dry).

Wash with TBS-T (5 min x 3).

Incubate with IgG Detector (1,000 - 4,000-fold dilution with IgG Detector Dilution Buffer*1) at room temperature for 1 hour.

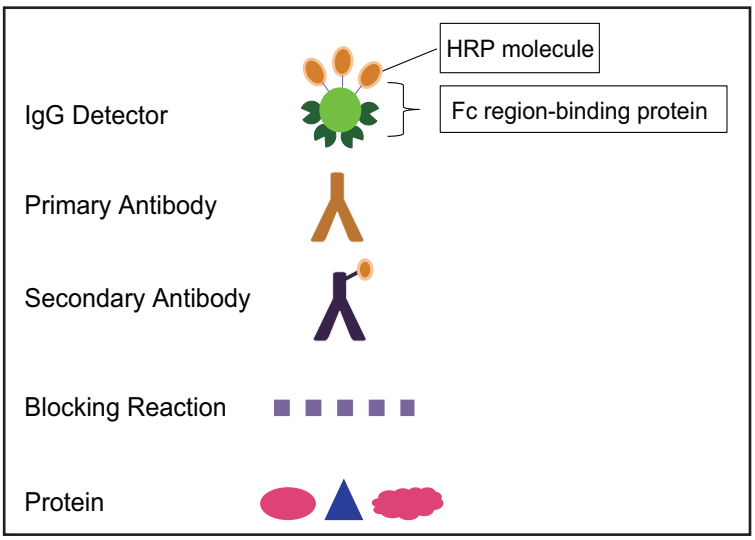
Wash with TBS-T (5 min x 5).

Detect with Western BLoT HRP Substrate series.

* 1 When Mouse IgG_1 is used as a primary antibody, Enhancer for Mouse IgG v2 is added to IgG Detector Dilution Buffer (1 : 2,000 dilution).

* 2 Multiple primary antibodies can be added in the primary antibody reaction solution.

* 3 When a protein molecular weight marker with IgG binding sites is used, add the Marker Detection Reagent v2 to the primary antibody reaction solution (1 : 10,000 dilution).



VI-1. Preparation and use of each reagent

a. Preparation of IgG Detector Dilution Buffer
This buffer is used to dilute IgG Detector Solution v2 (HRP labeled) in each protocol.

Dispense required amount of 10X Dilution Buffer v2 and dilute 10-fold with sterile water. Store at 4℃.

b. Preparation of Primary Antibody Dilution Buffer
This buffer is used to dilute the primary antibody in the VI-2b 2 Step High-Sensitivity Protocol and the VI-2c Secondary Antibody Sensitization Protocol.

Dilute 10X Dilution Buffer v2 10-fold with sterile water to prepare a 1X Dilution Buffer, then further dilute 10-fold with TBS-T to prepare a 1/10X Dilution Buffer.

c. Use of Enhancer for Mouse IgG v2
This reagent is essential when using a mouse IgG primary antibody.

1. Dilute Enhancer for Mouse IgG v2 2,000-fold by adding it to the required amount of IgG Detector Dilution Buffer (prepared in a.) and mix.
2. Use this solution instead of IgG Detector Dilution Buffer for dilution of IgG Detector Solution v2 (HRP labeled)

d. Use of Marker Detection Reagent v2
This reagent is used to detect protein molecular weight markers that have IgG binding sites (Thermo Fisher Scientific' MagicMark, etc.) at the same time as the target protein. Other molecular weight markers, which do not have IgG binding sites, cannot be detected.

Add Marker Detection Reagent v2 at 1 : 10,000 to the solution of primary antibody.

VI-2. Protocol details

VI-2a. Rapid Protocol

This is a quick detection protocol to perform Western blot detection in approximately 1 hour. It can also provide higher-sensitivity detection* by extending the incubation time*.

1. Perform SDS-PAGE.
2. Transfer proteins from an SDS-PAGE gel to a PVDF membrane, using an appropriate protocol.
3. Place the membrane in blocking buffer for 5 minutes* at room temperature.
4. Add the primary antibody and IgG Detector Solution v2 (HRP labeled) to IgG Detector Dilution Buffer at an appropriate dilution. React at first with the manufacturer's recommended concentration of the primary antibody, and optimize the concentration as necessary. Use IgG Detector at 1 : 2,000; The solution containing both the primary antibody and IgG Detector allow to stand 5 min before using.
   Note: When sodium azide is contained in the primary antibody, dilute so that the final concentration of sodium azide is less than 0.001%.
5. Incubate the membrane in the solution prepared in Step 4 for 30 minutes* at room temperature.
6. Wash the membrane with TBS-T (5 min x 5).
7. Perform antibody detection using a chemiluminescence substrate for HRP, such as the Western BLoT HRP Substrate series etc.

* By increasing the blocking time (Step 3) to 30 minutes and the primary antibody incubation time (Step 5) to 1 hour, higher detection is possible. With these changes, the required time for detection is ~2 hours.

VI-2b. 2 Step High-Sensitivity Protocol

This is the standard protocol for this product, and can provide higher sensitivity detection compared to detection using a labeled secondary antibody.

1. Perform SDS-PAGE.
2. Transfer proteins from an SDS-PAGE gel to a PVDF membrane, using an appropriate protocol.
3. Place the membrane in blocking buffer for 1 hour at room temperature.
4. Wash with TBS-T (5 min x 3).
5. Dilute the primary antibody to an appropriate dilution ratio with the Primary Antibody Dilution Buffer. Optimization of primary antibody dilution is required. The signal intensity will be increased when Western BLoT Immuno Booster Solution 1 in Western BLoT Immuno Booster (Cat. #T7111A, sold separately) is used as the dilution buffer.
6. Incubate the membrane in the diluted primary antibody solution for 1 hour at room temperature.
7. Wash the membrane with TBS-T (5 min x 3).
8. Dilute IgG Detector Solution v2 (HRP labeled) using the IgG Detector Dilution Buffer, and incubate the membrane in the diluted IgG Detector solution for 1 hour at room temperature. The dilution ratio of IgG Detector requires optimization at a range of 1,000 - 4,000-fold.
9. Wash the membrane with TBS-T (5 min x 5).
10. Perform antibody detection using a chemiluminescence substrate for HRP, such as Western BLoT HRP Substrate series, etc.

VI-2c. Secondary Antibody Sensitization Protocol

This protocol increases detection sensitivity by using IgG Detector after the usual secondary antibody reaction.

1. Perform SDS-PAGE.
2. Transfer proteins from an SDS-PAGE gel to a PVDF membrane, using an appropriate protocol.
3. Place the membrane in blocking buffer for 1 hour (room temperature).
4. Wash with TBS-T (5 min x 3).
5. Dilute the primary antibody to an appropriate dilution ratio with the Primary Antibody Dilution Buffer. Optimization of the dilution ratio is required. The signal intensity will be increased when Western BLoT Immuno Booster Solution 1 in Western BLoT Immuno Booster (Cat. #T7111A, sold separately) is used as the dilution buffer.
6. Incubate the membrane in the diluted primary antibody for 1 hour at room temperature.
7. Wash with TBS-T (5 min x 3).
8. Dilute the secondary antibody using an appropriate protocol, and incubate the membrane in the diluted secondary antibody solution for 1 hour at room temperature.
9. Wash with TBS-T (5 min x 3).
10. Dilute IgG Detector Solution v2 (HRP labeled) using the IgG Detector Dilution Buffer, and incubate with the membrane for 1 hour at room temperature. The dilution ratio of IgG Detector requires optimization within the range of 1,000 - 4,000-fold.
11. Wash with TBS-T (5 min x 5).
12. Perform detection using a chemiluminescence substrate for HRP, such as Western BLoT HRP Substrate series, etc.

VI-2d. Reprobe Sensitization Protocol

This protocol allows sensitive re-detection of a membrane which previously gave a weak signal by the standard method with a secondary antibody.

1. Prepare a membrane giving weak signal detection by the conventional method with a secondary antibody. Place the membrane in TBS-T to avoid drying; do not allow the membrane to dry.
2. Wash with TBS-T (5 min x 3).
3. Dilute IgG Detector Solution v2 (HRP labeled) using the IgG Detector Dilution Buffer, and incubate the membrane in the diluted IgG Detector for 1 hour at room temperature. The dilution ratio of IgG Detector requires optimization in the range of 1,000 - 4,000-fold.
4. Wash with TBS-T (5 min x 5).
5. Perform detection using a chemiluminescence substrate for HRP, such as Western BLoT HRP Substrate series, etc.

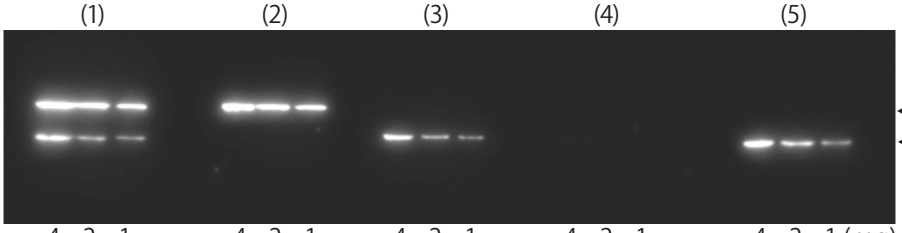
VII. Experimental Examples

β - Actin and GAPDH detection from HeLa cell lysate (VI-2b. 2 Step High-Sensitivity Protocol)

<Method>

Sample: HeLa cell lysate
Membrane: PVDF membrane (Pore size 0.45 μm)
Blocking buffer: Western BLoT Blocking Buffer (Protein Free) (PFB)
Primary antibody: Monoclonal Mouse Anti-β-actin (isotype IgG_1)
Mouse Anti-Glyceraldehyde-3-Phosphate Dehydrogenase (isotype IgG_1)
Secondary antibody: IgG Detector
Goat Anti-Mouse IgG (H+L) Peroxidase conjugated
Detection reagent: Western BLoT Rapid Detect v2.0
Western BLoT Chemiluminescence HRP Substrate (Cat. #T7101A)
Detection: CCD camera

		(1)	(2)	(3)	(4)	(5)
HeLa cell lysate		4, 2, and 1 μg/lane				
Blocking buffer		PFB				
Primary antibody, final concentration 0.5 μg/ml	Mouse anti-β-actin (IgG_1)	○	○	-	-	-
	Mouse anti-GAPDH (IgG_1)	○	-	○	○	○ PFB dilution
Secondary antibody, final concentration 0.16 μg/ml	IgG Detector	IgG Detector + Enhancer for Mouse IgG v2			IgG Detector	-
	Secondary antibody	-	-	-	-	○ PFB dilution



< Results >

Sensitivity of β -actin and GAPDH detection

	(1)	(2)	(3)	(4)	(5)
β-Actin	< 1.0 μg	< 1.0 μg	-	-	-
GAPDH	1.0 μg	-	1.0 μg	Undetectable or > 4.0 μg	1.0 μg

Based on results (3) and (5), Western BLoT Rapid Detect v2.0 can be used to obtain equivalent results as conventional Western blot methods using a secondary antibody.
Based on results (1), (2), and (3), simultaneous antigen detection provides a similar level of detection with the single antibody detection method.
Based on results (3) and (4), Mouse IgG_1 antibody can be detected with this kit by adding Enhancer for Mouse IgG v2.

VIII. Troubleshooting

Since Western blotting is a multi-step process, optimization of conditions are required. Perform preliminary experiments to determine the suitable amount of protein amount to be used, the dilution ratio of IgG Detector and primary antibody, etc.

Problem	Cause	Solution
High background	Concentration of antibody used was too high.	Increase the dilution ratio of antibody used to reduce its concentration.
	Concentration of IgG Detector was too high.	Increase the dilution ratio of IgG Detector to reduce its concentration.
	Insufficient blocking.	Optimize blocking conditions.
	Incubation with antibody was too long.	Shorten antibody incubation time.
	Insufficient washing.	Increase the volume of washing buffer, and the number and duration of washes. Background can be high without sufficient washing, especially when Immuno Booster is used as a primary antibody dilution buffer.
No band or weak band	Amount of antibody used was insufficient .	Decrease the antibody dilution ratio.
	Amount of antigen used was Insufficient .	Increase the amount of antigen.
	The membrane did not have enough transferred proteins.	Optimize the transfer conditions.
	Primary antibody was Goat IgG, Human IgG_3, or fragmented antibody (except mouse antibody).	Use the Secondary Antibody Sensitization Protocol.
	Primary antibody was Mouse IgG_1 or fragmented antibody (mouse antibody).	Add Enhancer for Mouse IgG v2 to IgG Detector dilution buffer.
	IgG Detector adsorbed.	Use a tube with low protein adsorption when dilution is performed using IgG Detector dilution buffer.
Many extra bands	Concentration of antibody used was too high.	Increase the antibody dilution ratio and reduce the concentration of antibody.
	Insufficient washing.	Increase the washing buffer volume, number of washes, and duration of washes.
	Insufficient blocking.	Optimize blocking conditions.
	IgG Detector was degraded.	IgG Detector deteriorates when it is stored at 4℃ or room temperature for a long time.
	The sample was derived from serum.	Sometimes extra bands are seen around 50 kDa, when the sample contains serum. Because these bands are derived from a reaction with IgG in serum, remove the IgG in serum using a Protein A column, etc.
Not detectable by the rapid protocol	Primary antibody was low purity.	When you use a primary antibody with low purity, such as anti-serum, sometimes a target band is not detected. Purify the antibody or switch to the 2 Step High-Sensitivity Protocol.
When simultaneous detection is performed with multiple primary antibodies, there is a weaker band than with standard detection.	Insufficient washing after primary antibody incubation.	Increase the number and duration of washes.
	Reactivity was different for multiple primary antibodies.	When detection is performed with multiple primary antibodies simultaneously, the reactivity can be different between each primary antibody and IgG Detector. Increase the concentration of the primary antibody giving a weak signal.

IX. Related Products

＜ Western BLoT HRP Substrate Series ＞
Western BLoT Chemiluminescence HRP Substrate (Cat. #T7101A/B)
Western BLoT Quant HRP Substrate (Cat. #T7102A/B)
Western BLoT Hyper HRP Substrate (Cat. #T7103A/B)
Western BLoT Ultra Sensitive HRP Substrate (Cat. #T7104A/B)

＜ Western Blot Chemiluminescence Enhancer ＞
Western BLoT Immuno Booster (Cat. #T7111A)
Western BLoT Immuno Booster PF (Cat. #T7115A)

＜ Blockng Buffer ＞
Western BLoT Blocking Buffer (Protein Free) (Cat. #T7132A)
Western BLoT Blocking Buffer (Fish Gelatin) (Cat. #T7131A)

＜ Buffer Tablets and Powders ＞
Tris-Glycine-SDS Buffer (TG-SDS) Powder, pH 8.3 (Cat. #T9101)
Tris-Glycine Buffer (TG) Powder, pH 8.3 (Cat. #T9102)
Tris Buffered Saline (TBS) Tablets, pH 7.6 (Cat. #T9141)
Tris Buffered Saline with Tween20 (TBS-T) Tablets, pH 7.6 (Cat. #T9142)
Phosphate Buffered Saline (PBS) Tablets, pH 7.4 (Cat. #T9181)
Phosphate Buffered Saline (PBS) Tablets without Potassium, pH 7.4 (Cat. #T9182)
Phosphate Buffered Saline with Tween20 (PBS-T) Tablets, pH 7.4 (Cat. #T9183)

<Protein Quantitative Kit >
TaKaRa BCA Protein Assay Kit (Cat. #T9300A)
TaKaRa Bradford Protein Assay Kit (Cat. #T9310A)