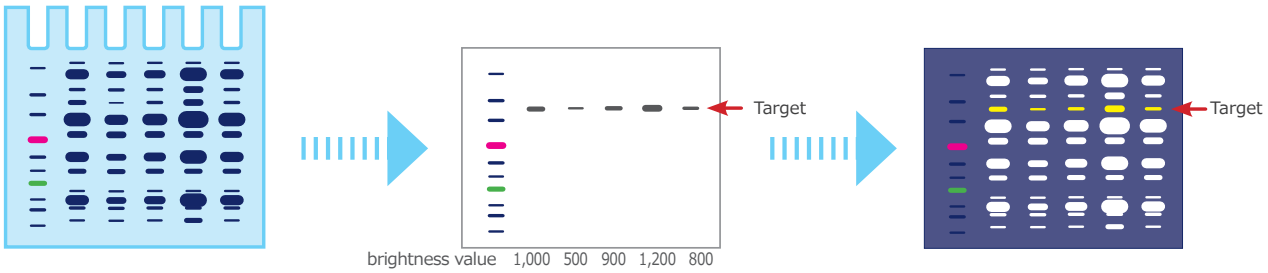
How to Normalize by Total protein

1. Overview

Western blotting is the most commonly used method to detect target proteins. In order to detect changes in the expression of a target protein or to compare expression levels between samples, the amount of the target protein must be quantified in some way. In general, Western blotting data are subject to variations among samples and lanes. Therefore, in order to minimize these effects, a certain index (reference) is used to correct the data (normalization). In the past, housekeeping protein (HKP) expression levels have been used as a reference for normalization. Recently, however, it has been pointed out that the expression levels of housekeeping proteins are not necessarily constant in different tissues, cell types, cell cycles, or developmental stages, and that the expression levels of housekeeping proteins are also far from those of target proteins. It is now said that it is necessary to be very careful when using them for normalization. In such a situation, total protein (TP, total protein) has been attracting attention as a reference that can be used for normalization instead of housekeeping proteins.
In this issue, we will introduce a method of data normalization using total protein with Ato products and others.

Detect Target Protein



① Electrophoresis

What proteins are in the sample?

When electrophoresis is performed, the proteins in the sample are divided into bands. However, it is not possible to determine which band is the target protein.

② Western blotting

Is the target protein expressed?

Western blotting gives the band and amount (brightness value) of the target protein. The molecular weight of the target protein can also be estimated from the mobility of the molecular weight marker. However, it is not possible to determine in which sample the protein is most abundantly expressed.

③ Data analysis

Which band is the target protein?

Overlaying the electrophoresis pattern with the Western blotting detection bands, it is possible to determine which band is the target protein. Using the information on the total protein in each lane as a reference, it is also possible to analyze how much of the protein is contained in the sample and in which sample it is most abundantly expressed.

Why is normalization necessary?

① Heterogeneity of the sample itself
Differences by species, tissue and developmental stage, and extraction methods can affect the results.

② Non-uniformity of applique volume
It is affected by the method of protein quantification, the extraction solvent, and the technique used for appling.

③ Heterogeneity in transcription efficiency, detection efficiency, and antibody reaction
It is affected by the transfer buffer, transfer method, technique, antibody titer, dilution rate and reaction time.

④ Comparison among multiple blots
A standard is needed when comparing the expression levels of multiple blots, such as when comparing multiple specimens.

Normalization is necessary to minimize these effects

For normalizing references?

① Total protein

- ◆ **Labeled with fluorescent dyes such as EzLabel FluoroNeo, Cy5**
- ◆ Using stain free gel
- ◆ Staining of blotting membranes (CBB, Ponceau, etc.)

② House keeping protein (HKP)

- ◆ Expression levels of GAPDH, actin, tubulin, etc.

Data normalization corrects the expression levels of the target protein by reference

However, the following precautions are said to be necessary when using housekeeping proteins as references.
The expression level of the reference should not be affected by the experimental conditions.
The expression levels of the reference and the target protein should not be far apart.
The detection of the reference should not interfere with the detection of the target protein.

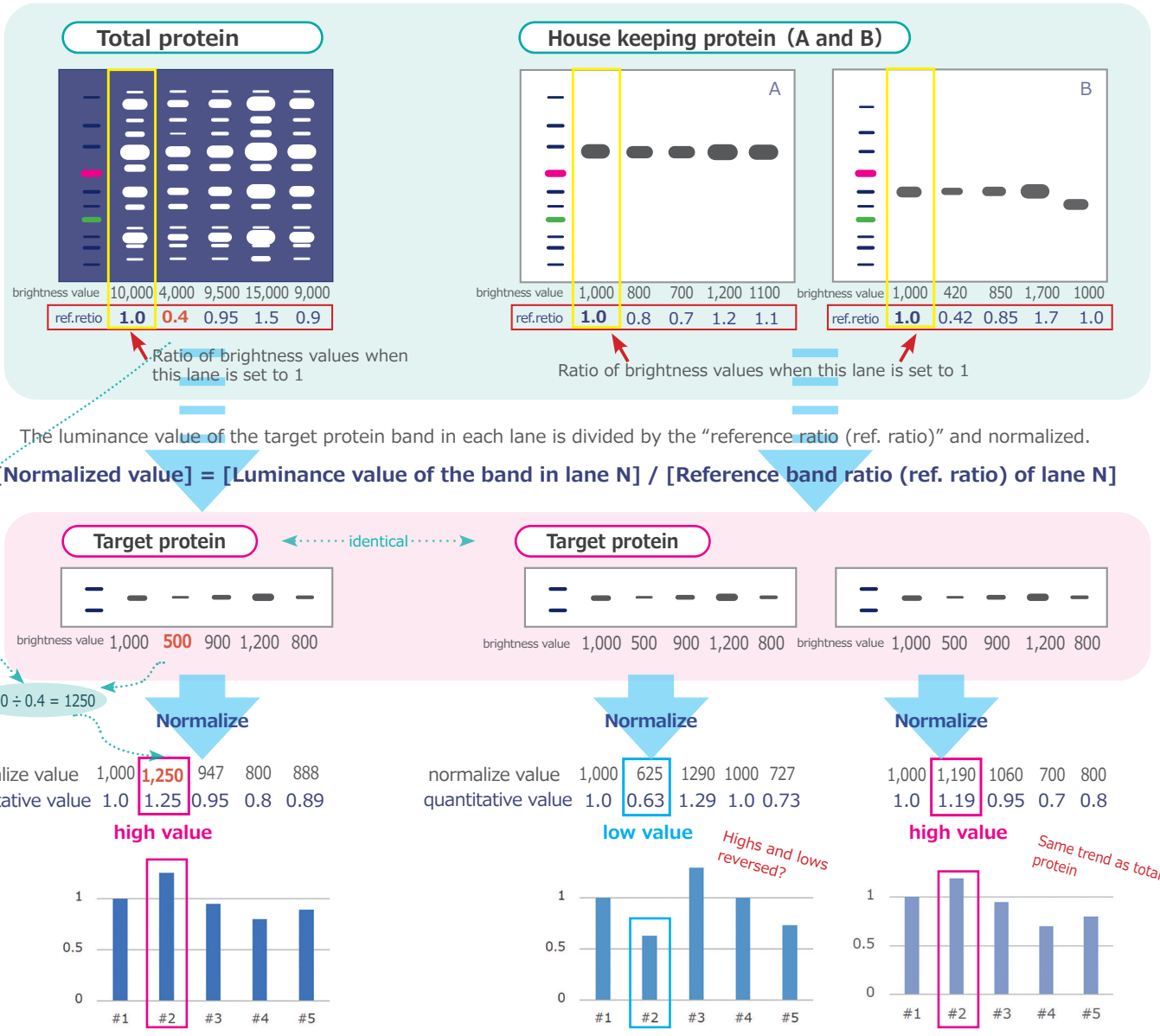
2. What is normalization?

As mentioned above, protein samples are heterogeneous, and even if the same amount of the same sample is applied and electrophoresed, there will be differences between lanes. No matter how much care is taken, uneven transcription and variations in antibody reaction are inevitable. Therefore, the process of normalization is essential for accurate, reproducible, and quantitative Western blotting. In Western blotting normalization, the brightness value of the target protein is divided by the relative value of each lane calculated from the brightness value of the reference (reference ratio).

Normalized quantification

The ratio of the reference luminance values between lanes (the "reference ratio") is calculated and used as a correction factor for variation.

[Reference ratio (ref. ratio)] = [luminance value of each reference] / [luminance value of the reference in the first numbered lane].



① **Normalization by total protein**

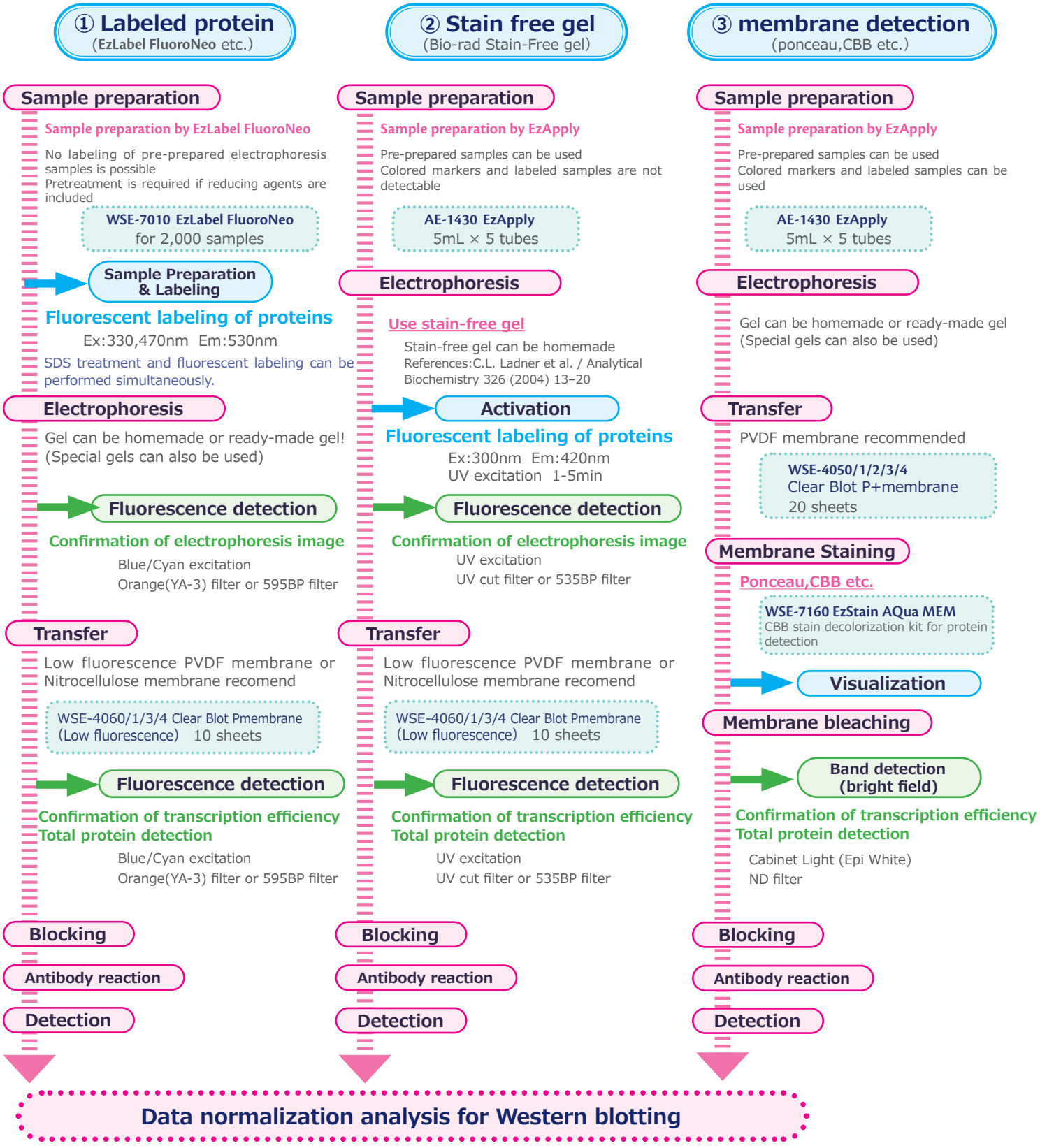
This is an example of normalizing the amount of protein transferred to the PVDF membrane (total protein) as a reference. Total protein is a universal indicator because it is not the expression level of a specific protein. It minimizes the effects of antibody reactions, uneven transcription, and tissue differences. The above shows an example where the sample with the weakest target protein signal is the most abundantly expressed as a result of normalization.

② **Normalization by housekeeping proteins**

This is an example of normalizing housekeeping proteins such as GAPDH and actin as reference. Although housekeeping proteins are relatively stably expressed, they may not be accurately corrected if their expression levels vary from tissue to tissue, if their expression levels are significantly higher than the target protein, or if they are detected in excess due to good antibody reactivity. Above is an image of an example where housekeeping proteins are excessively detected (A) and an example where they are ideally detected (B). This is an extreme example, but it shows the possibility of reversal between samples with high and low expression levels.

3. Experiment Flow

The figure below shows the experimental flow for total protein normalization. In the case of total protein normalization, it is necessary to visualize the protein bands on the transcribed PVDF membrane. there are three major methods for detecting proteins on the PVDF membrane: (1) sample labeling, (2) stain-free gel, and (3) post-transcription membrane staining.

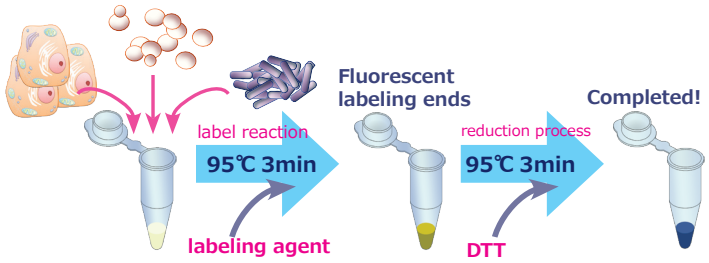


In this article, we will explain the experimental methods for (1) sample labeling and (2) stain-free gel. In addition, we will briefly introduce the normalization analysis of Western blotting results using Ato' s image analysis software CS Analyzer 4.

4. Gel stain free with sample labeling

4-1.Sample preparation (EzLabel FluoroNeo)

EzLabel FluoroNeo can be used in place of SDS sample buffer for fluorescent labeling of proteins and preparation of samples for electrophoresis. After electrophoresis, the gel does not need to be stained or decolorized, and bands can be detected immediately.



Experimental materials : Tissues, cells, bacteria, etc.
EzLabel FluoroNeo (WSE-7010)
Micro centrifuge tubes, tips, etc.
Centrifuge, micropipettes, etc.

Experimental procedure :

(1) Prepare electrophoresis samples using EzLabel FluoroNeo. 40 µL of protein sample is mixed with 10 µL of Sample buffer (5x conc.) and 0.5 µL of Labeling reagent supplied with the kit.

(2) Heat at 95° C for 3 minutes (boiling is also OK).

(3) Add 2 µ L of Reducing agent (DTT) to the mixture in (2) and mix.

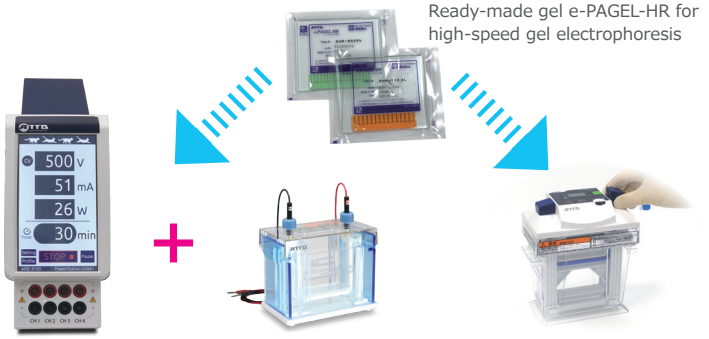
(4) Heat at 95° C for 3 minutes (boiling is also OK).

The prepared sample can be stored at -20° C in a light-shielded container.

*The presence of reducing agents in the sample can significantly interfere with the fluorescent labeling reaction. Therefore, perform the reduction process (Steps (3)-(4)) after labeling.

4-2.Electorophoresis

Electrophoresis samples labeled with EzLabel FluoroNeo can be separated using common gels, buffers, and electrophoresis conditions. Here we introduce high-speed electrophoresis using ready-made gels (ePAGEL-HR).



Power Supply
WSE-3100
PowerStation Ghibli I

WSE-1165 m-PAGE
Electrophoresis apparatus

Electrophoresis System with Power Supply
WSE-1150 PageRun Ace

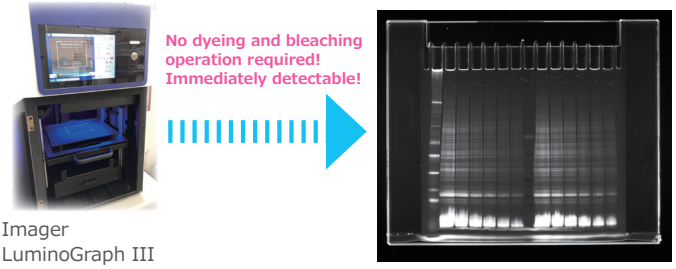
Experimental materials :
Samples prepared with EzLabel FluoroNeo
Precast gel (e-PAGEL-HR etc.)
Electrophoresis buffer (AE-1410 EzRun etc.)
Electrophoresis equipment, power supplies, chips, etc.

Experimental procedure :

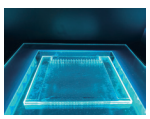
(1) Set ePAGEL-HR on PageRun Ace (electrophoresis system with power supply).
(2) Apply 5 to 10 µ L of sample per lane.
The appropriate sample concentration is 100 ng to 1 µ g/lane for purified protein and 1 to 50 µ g/lane for extracted solution.
(3) Press the start button to begin electrophoresis.
The electrophoresis is performed for about 35 minutes in High mode (24W) or about 80 minutes in Standard mode (20mA/gel). If an external power supply is used, the gel electrophoresis is performed at 300 V for about 35 minutes or at 150 V for about 75 minutes.
(4) When the swim tip (dye line) reaches 5-10 mm above the bottom edge of the gel, stop the output and terminate the gel electrophoresis.
*If the sample solvent contains amino groups such as Tris, it may cause nonspecific signals (which will disappear by washing the gel with distilled water).

4-3.Confirmation of electrophoresis pattern (fluorescence detection)

After electrophoresis with EzLabel FluoroNeo, the gel can be immediately excited with Blue LED or Cyan to see the band patterns.



Imager
LuminoGraph III



Cyan Light source
CyanoView

Gel: EHR-T520L
Sample: 1/2 dilution series of HeLa and HepG2 cell extracts
Imager: LuminoGraph III
Conditions : Blue LED Light source
BPF595 filter
exposure time 1sec

Experimental materials :
Gel after electrophoresis (using EzLabel FluoroNeo)
LuminoGraph III Lite or LuminoGraph II EM
Luminograph I CMOS etc.
Cyan (CyanoView) ,BlueLED

Experimental procedure :

(1) Remove the gel from the swimming bath after the swimming is finished. Without removing the gel plate, wash the glass surface lightly with tap water and wipe off the water with a paper towel.

(2) Prepare the photographic equipment, light source, and filter.

Imager	Light source	filter
LuminoGraph I CMOS	CyanoView	YA-3 (560LPF)
LuminoGraph II EM LuminoGraph III Lite	Blue LED or CyanoView	BPF525/YA-3 BPF595/535

[Ex: 330 (UV), 470 nm, Em: 530 nm]

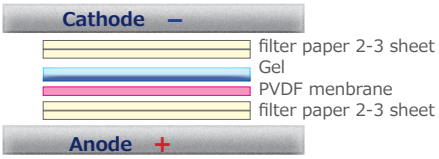
(3) Place the entire plate of gel on the light source illuminated surface and take a picture.

4-4.Transfer

The use of a PVDF film for fluorescence detection with low autofluorescence is desirable, but a general PVDF film can also be used for detection. Especially when using a Blue LED light source, the effect of autofluorescence can be reduced. In this section, we introduce the use of a fast transfer buffer (EzFastBlot) for fast transfer.

Powered BLOT 2M

Setting



Transcription Buffer and Transcription Conditions

	EzBlot	EzFastBlot	EzFastBlot HMW	EzRun TG (Towbin)
Cat#	AE-1460	AE-1465	WSE-7210	WSE-7055
Transfer condition	2 mA/cm^2 60 min (~40V)	20~25V c.v. 5~15 min (500mA/gel)	25V c.v. 15~30 min (500mA/gel)	2 mA/cm^2 60 min (~40V)

Experimental materials :

Gel after electrophoresis (using WSE-7010 EzLabel FluoroNeo)
Transfer buffer (AE-1465 EzFastBlot, etc.)
PVDF membrane (ClearBlot P membrane (low fluorescence), etc.)
Blotting equipment (PoweredBLOT 2M, etc.)
Centrifuge, micropipette, etc.

Experimental procedure :

(1) Hydrophilize the PVDF membrane with methanol in advance (5 sec) and immerse it in EzFastBlot (shake for at least 15 minutes).

(2) Wash the gel after fluorescence detection with EzFastBlot, and stack 2 or 3 sheets of filter paper (0.9 mm thick), PVDF membrane, gel, and 2 or 3 sheets of filter paper in this order, starting from the anode side (bottom). Immerse the filter paper thoroughly in EzFastBlot immediately before layering.

*Air is pushed out using a special roller to make the filter paper, membrane, and gel adhere to each other.

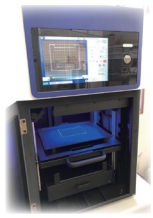
(3) Press the start button to begin transfer.

The system is energized in Rapid mode for 10 to 15 minutes.

* When using an external power supply, transfer at 24 V for about 10 to 15 minutes or at 12 V for about 30 minutes.

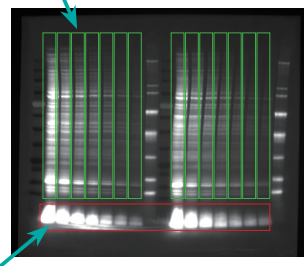
4-5.Confirmation of transcription efficiency (fluorescence detection)

The fluorescence intensity of EzLabel FluoroNeo correlates with the protein concentration, so the signal intensity of the protein bands on the PVDF membrane can be used for normalization as total protein. The signal intensity of the protein bands on the PVDF membrane can be used for normalization as the total protein. See “6. Normalization by Total Protein” on page 8 for more information on normalization.



Imager
LuminoGraph III

Signals for all bands in each lane
⇒ Total Protein



Nonspecific signals derived from foreign substances or amino acids in the sample

Can be removed by TCA precipitation
Can be reduced by rinsing

PVDF : Clear Blot P+membrane
Imager : LuminoGraph III
Conditions : BlueLED light source
BPF595 filter
exposure time 0.1 sec

Experimental materials :

PVDF film after transfer (using EzLabel FluoroNeo)
LuminoGraph III Lite or Luminograph II EM
Luminograph I CMOS etc.
Cyan (CyanoView) ,BlueLED

Experimental procedure :

(1) Lightly wash the PVDF membrane with distilled water after transfer is completed.

(2) Prepare the photographic equipment, light source, and filters.

Imager	Light source	filter
LuminoGraph I CMOS	CyanoView	YA-3 (560LPF)
LuminoGraph II EM LuminoGraph III Lite	Blue LED or CyanoView	BPF525/YA-3 BPF595/535

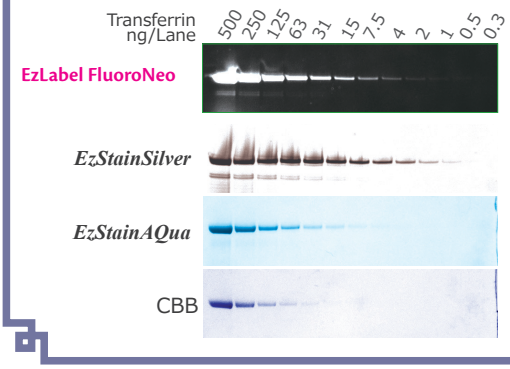
(3) Place the PVDF film on the light source irradiated surface and take a picture.

*Please handle the PVDF film with great care to avoid contamination. Place it between the Pitatto Clear (2322438) to protect it from contamination without affecting the fluorescence detection.

The dryer the PVDF film is, the stronger and clearer the fluorescence signal can be detected. Dry PVDF membranes should be hydrophilized with methanol before proceeding to blocking and antibody reaction.

Related Products

Detection sensitivity of EzLabel FluoroNeo



- ✓ **Sample preparation & fluorescent labeling**
- ✓ **Gel stain free ministry**
- ✓ **Fluorescence detection immediately after electrophoresis!**
  Compatible with Cyan, Blue LED, UV light sources
  Ex: 330 (UV), 470 nm, Em: 530 nm
- ✓ **Silver stain level detection sensitivity**
  No change in band mobility after labeling

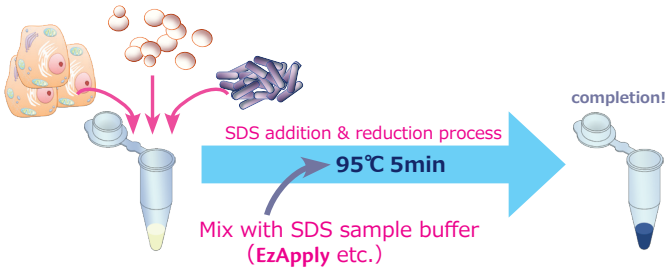
Western blotting available

Product	EzLabel FluoroNeo
type/code	WSE-7010/2332333
Kit Contents	Sample buffer (5x): 12 mL Labeling reagent: 10 mg Reducing agent (DTT): 300 mg MW marker : 600 µ L RIPA Lysis buffer: 10 mL
Storage	Frozen -20° C 1 year (unopened)

5. Stain-free gel

5-1. Sample Preparation (EzApply)

When using stain-free gels, electrophoresis samples should still be prepared in the usual manner. Since colored markers cannot be detected, colorless markers should be used in combination.



Experimental materials : Tissues, cells, bacteria, etc.
SDS sample buffer (AE-1430 EzApply)
Micro centrifuge tubes, tips, etc.
Centrifuge, micropipettes, etc.

Experimental procedure :

(1)Add 50 μ L of EzApply (2 × concentration, DTT added) to 50 μ L sample and mix.

(2) Heat in a small block incubator WSC-2610 MyMiniBlock at 95° C for 5 minutes (boiling is also acceptable).

(3) Centrifuge at 15,000 rpm for 5 minutes (or not) and collect the supernatant.

*Prepared samples can be stored at -20° C.

5-2.Electrophoresis

Use a stain-free gel for the electrophoresis. When using precast gels, follow the instruction manual. Here, we will show you how to make your own stain-free gel and perform the gel electrophoresis.

For details on how to make stain-free gels, please refer to the following article by Ladner et al.

References
C.L. Ladner et al., Analytical Biochemistry 326 (2004) 13–20
Visible fluorescent detection of proteins in polyacrylamide gels without staining

Gel Composition Table	Separation Gel				Concentrated Gel
Gel concentrate	7.5%	10 %	12.5%	15%	4.5%
D.W.	5 mL	4.2 mL	3.3 mL	2.5 mL	3 mL
30% A.A/BIS*	2.5 mL	3.3 mL	4.2 mL	5 mL	0.75 mL
EzGelAce	2.5 mL	2.5 mL	2.5 mL	2.5 mL	1.25 mL
TCE**	0.05 mL	0.05 mL	0.05 mL	0.05 mL	0.05 mL
10% APS	0.075 mL	0.05 mL	0.05 mL	0.05 mL	0.05 mL
TEMED	0.005 mL	0.005 mL	0.005 mL	0.005 mL	0.005 mL

*30% A.A/BIS: 30(w/v)% acrylamide/bis(29:1) solution
**TCE: 2,2,2-trichloroethanol (CAS No.: 115-20-8)
The composition table above is the volume required per minigel.

Experimental materials : Phoresis Samples
Stain-free gel
Phoresis buffer (AE-1410 EzRun, etc.)
Electrophoresis equipment, power supply, chips, etc.
(If you make your own)
30(w/v)% acrylamide/bis(29:1) solution
Gel buffer solution (WSE-7310 EzGelAce)
Ammonium persulfate (APS)
TEMED

Experimental procedure :

(1) Assemble the gelator. Follow the instruction manual of the gel processor to be used for assembly.

(2) Prepare gel solutions of separated gel and concentrated gel by mixing reagents other than APS and TEMED, referring to the gel composition table on the left. Gels are prepared by adding APS and TEMED just before the start of polymerization.

(3) Set the gels in PageRun Ace (electrophoresis system with power supply).

(4) Apply 5-10 μ L of sample per lane.

*Sample concentration of 100 ng to 1 μ g/lane for purified protein and 1 to 50 μ g/lane for extract is appropriate.

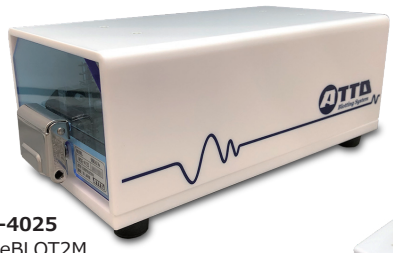
(5) Press the start button to begin electrophoresis.

The electrophoresis is performed in High mode (24W) for about 35 minutes or in Standard mode (20mA/gel) for about 80 minutes.

If an external power supply is used, the gel electrophoresis is performed at 300 V for about 35 minutes or at 150 V for about 75 minutes.

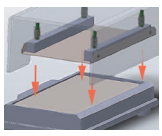
(6) When the tip of the gel (dye line) reaches 5-10 mm above the bottom of the gel, stop the power output to finish the gel electrophoresis.

Related Products



WSE-4025
HorizeBLOT2M

4-point spring method



Easier with power supply!

WSE-4125
PoweredBLOT 2M

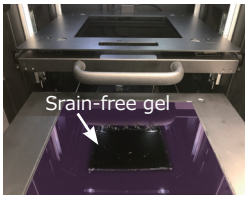
HorizeBLOT 2M/4M

- ✓ **Strong and even pressure with 4-point spring system to fix gel & membrane tightly**
- ✓ **Minimized risk of transfer flow and unevenness**
- ✓ **High-speed transfer in as little as 5 minutes (2 mini-gels, 1 wide gel)**
- ✓ **Improved operability and data reproducibility**

	HorizeBLOT 2M	HorizeBLOT 4M
Type / code	WSE-4025 / 2322466	WSE-4045 / 2322476
Gel type	Mini-gel x2 , Wide-gel x1	Mini-gel x4 , Wide-gel x2
Electrode size	205(W)x100mm(H)	205(W)x200mm(H)
Electrode	Anode : Corrosion-resistant platinum-plated titanium plate Cathode : Stainless steel	
size/weight	246x145x92mm / 1.8kg	246 x235x92mm / 3kg

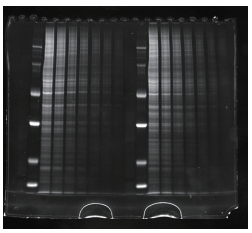
5-3.Detection

After electrophoresis, the gel is not immediately fluorescently detectable. To visualize proteins, place the gel directly on a UV irradiation device and irradiate it for about 1 min. 312 nm UV wavelength is used. Once visualized by UV irradiation, the fluorescence of the gel is relatively stable, so rinse the gel with distilled water to condition it before photographing to obtain a clean image and confirm the electrophoresis pattern.



UV irradiated surface of the LuminoGraph III

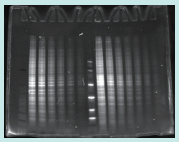
Labeled by UV irradiation!



Gel: 10% Stain-free gel(home made)
sample: 1/2 dilution series of HeLa and HepG2 cell extracts
Imager: LuminoGraph III
Condition : UV transilluminator
BPF535 filter
Exposure time 2sec

reference

Precast gel swimming pattern samples and imaging conditions are equivalent to those shown on the right.



Danger Direct exposure to UV radiation may cause blindness and skin irritation. Use protective eyewear, gloves, and protective clothing.

Experimental materials :

Gel after electrophoresis (usiing stain-free gel)
LuminoGraph III Lite or Luminograph II EM
[UV transilluminator(312nm) ,filter]

Experimental procedure :

Gel visualization

(1) Wash the glass surface of the gel lightly with water after the end of swimming without removing the gel plate, and wipe off the water with a paper towel.

(2) Drop 1~5mL of distilled water onto the UV irradiator.

(3) Remove the gel from the plate and place it on top of the drop of water on the UV irradiation device.
Do not rinse or wash the gel. The signal will be weakened.
*Different UV wavelengths may cause the gel not to be visualized.
*Please be careful not to look directly at or touch the UV irradiation light source.
*Because the process is for visualizing the gel, there is no need to prepare and place the gel on the irradiated surface. Also, please note that the gel is easily torn.

(4) Turn on the UV irradiation device and irradiate the gel for about 1 minute.

(5) After UV irradiation, rinse with distilled water or the like to condition the gel.

Gel documentation

(6) Set the UV light source and filter of the photographic equipment.

Imager	Light source	filter
LuminoGraph II EM LuminoGraph III Lite	UV (312nm)	BPF525/535

(7) Place the gel on the UV-irradiated surface or on the dedicated tray and photograph.
*Clean the UV-exposed surface before photographing.
*Water stains on the UV-exposed surface can be removed by using aqueous citric acid solution.

5-4.Transfer

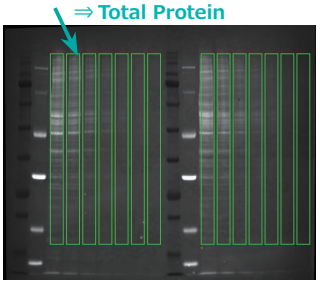
Use a PVDF membrane for fluorescence detection with low autofluorescence. See “4-4. Transfer” on page 5 for transfer method.

5-5. Confirmation of transcription efficiency (fluorescence detection)

The stain-free gel with visualization can detect the bands on the PVDF membrane after transfer as well as the gel, and the signal intensity of the protein bands on the PVDF membrane can be used for normalization as a total protein. For more information on normalization, please refer to “6. Normalization by Total Protein” on page 8.

Imager
LuminoGraph III

Signals for all bands in each lane
⇒ Total Protein



PVDF: Low fluorescence PVDF
imager: LuminoGraph III
condition : UV transilluminator
BPF535 filter
Exposure time 1sec

Experimental materials :

PVDF film after transfer (using stain-free gel)
LuminoGraph III Lite or Luminograph II EM
[UV transilluminator(312nm) ,filter]

Experimental procedure :

(1) Lightly wash the PVDF membrane with distilled water or other solution after the transfer is completed.

(2) Prepare UV light source and filters for imaging equipment.

Imager	Light source	filter
LuminoGraph II EM LuminoGraph III Lite	UV (312nm)	BPF525/535

(3) Place the PVDF film on the UV-irradiated surface or on a dedicated tray and photograph.

*Please handle the PVDF film carefully to avoid contamination.

*When excited by UV irradiation, the wetter the PVDF film is, the lower the background becomes and the clearer the bands can be detected. Please be careful not to let it dry out during imaging.

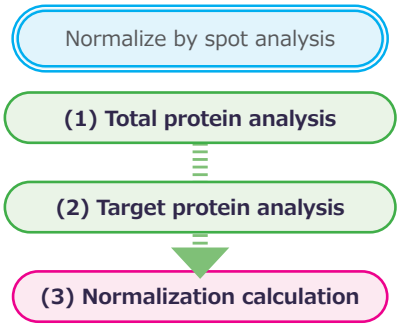
6. Normalization method by total protein

6-1. Normalization by total protein

Normalize the total protein detected using EzLabel FluoroNeo or stain-free gels as a reference. The reference is the total amount of transcribed protein (brightness value) for each lane on the PVDF membrane.

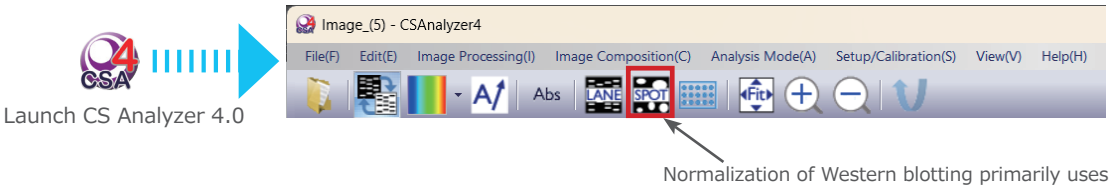
6-2. Normalization Procedure

Whether using EzLabel FluoroNeo or a stain-free gel, the images are analyzed in the same way. The images required for normalization are the bands of the total protein transferred onto the PVDF membrane (fluorescence image or bright field image if dye-stained) and the bands of the target protein after antibody reaction (luminescence image or fluorescence image, etc.). First, the signal intensity of the total protein band in each lane on the PVDF membrane is quantified, followed by the signal intensity of the target band. Here, we introduce the analysis method using the image analysis software CS Analyzer 4. The analysis procedure is as follows: (1) to (3).
(1) Spot analysis of total protein detection image on PVDF membrane
(2) Spot analysis of target protein detection image
(3) Normalization calculation



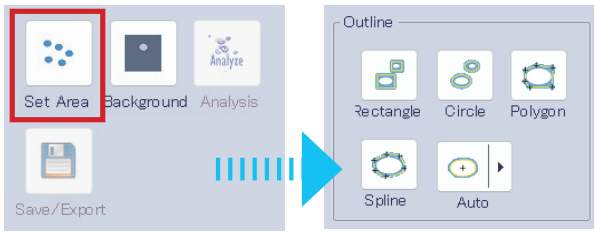
6-3. Total protein analysis

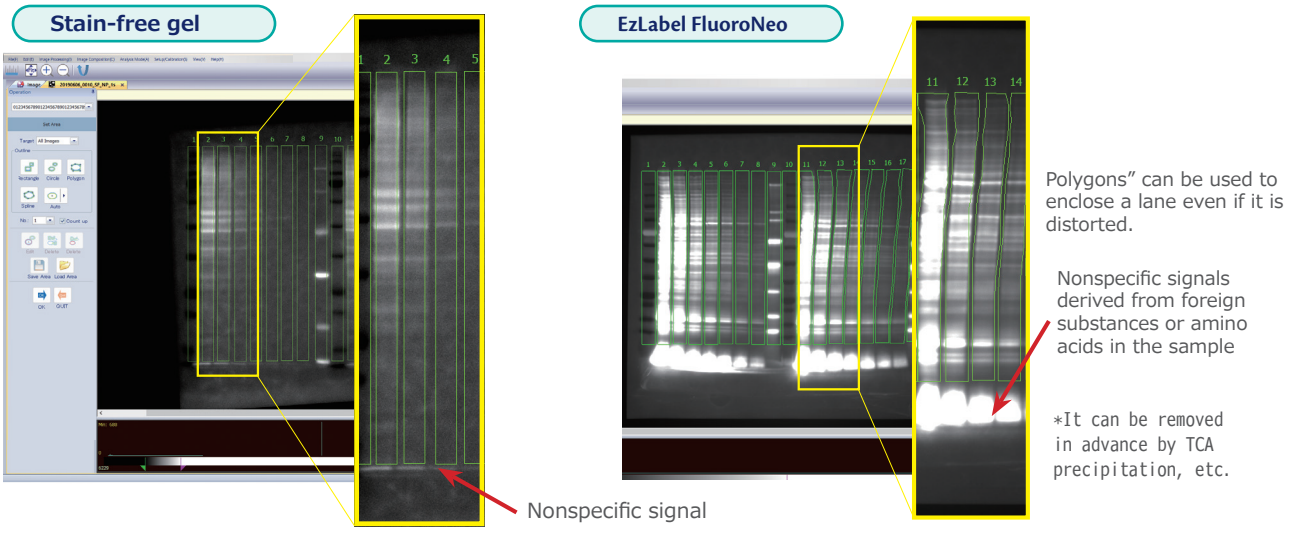
First, spot analysis is performed on the fluorescence imaging images in which the total protein is detected as a reference for normalized conversion.



(1) Outline each lane

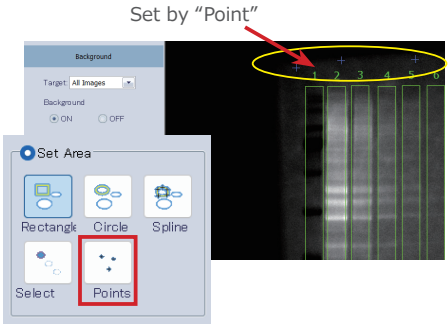
Select “Area Settings” - “Square” or “Polygon” to enclose the outline of each lane. If the lane is curved, “Polygon” is more accurate.
When outlining a lane, do not include the swim tip. The tips contain nonspecific signals from dyes such as BPB and do not necessarily reflect the total protein content. Especially when labeled with EzLabel FluoroNeo, if the gel concentration is high, non-specific signals derived from foreign substances or amino acids in the sample will be detected at the tip, so exclude these areas and enclose them.
When normalizing analysis with CS Analyzer 4, the analysis data of the reference protein must match the lane number of the target protein.





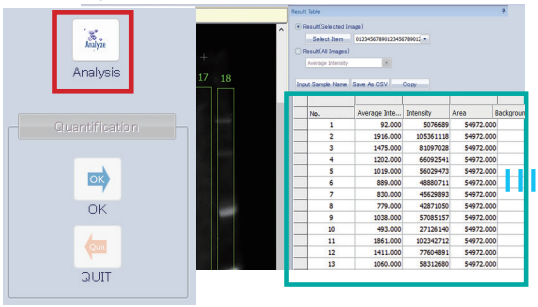
(2) Backgraund setting

Set background in the margins of the PVDF film other than the area enclosed in (1). You can use “Rectangle” or “Select Area” to set the same area as the spot, or “Point” to set multiple points in the margins other than the lanes.

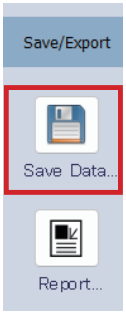


(3) Analysis and data storage

Click the “Analyze” button to quantify the signal intensity of the band, and then click the “Save Analysis Information” button to save the analysis results. The analyzed data can also be output in CSV format.



Measurement results are displayed.
Copy & paste or output in CSV format and save analysis data



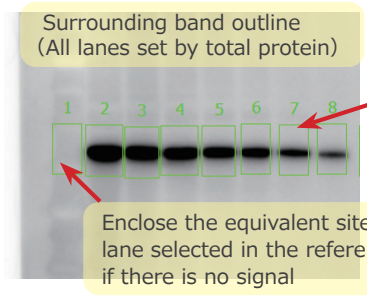
Whenever data is used for normalization, be sure to save the analysis information.

6-4. Target protein analysis

Next, spot analysis is performed on the luminescence (fluorescence, etc.) imaging images in which the target protein is detected by antibody reaction. The analysis method is the same as the total protein analysis method.

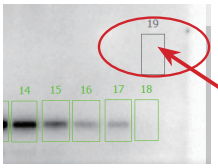
(1) Surrounding band outline

Select a “square” or “polygon” to enclose the outline of the target protein band by spot analysis. Especially for luminescence images, the intensity and size of the bands are affected by the contrast, so surround the bands with a certain degree of contrast. In addition, the lane number set in the total protein analysis should be the same as the band number of the target band to be analyzed. If the target band number and the lane number to be normalized are not the same, the data from another lane with the same number as the band number will be incorrectly corrected.



(2) Background Settings

Set background in the PVDF film margins other than the area enclosed in (1). Use “square” or “polygon” to enclose the same area as the spot, or use “point” to set multiple points in the margins other than the lane.



Related Products

LuminoGraph III Lite

High-end chemiluminescence imaging equipment

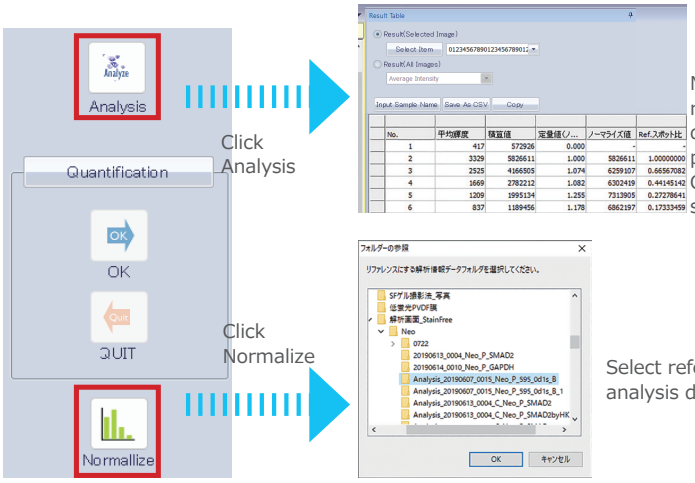
- ✓ Ultra-sensitive and high-resolution 6 megapixel cooled CCD camera
- ✓ Capable of capturing a wide variety of samples such as fluorescence, luminescence, visible light, etc.
- ✓ Various shooting modes such as auto exposure, totalizing, etc.
- ✓ Flat field distortion correction and dark image processing

名称	WSE-6370H LuminoGraph Ⅲ Lite
camera	Cooled CCD camera 2750 × 2200 pixel
lens	F0.8 manual
control	Windows PC
Fluorescence excitation light source	Epi LED : Blue(472nm)/Green(528nm)/Red(615nm) UV transilluminator : 312nm
white light	LED White Light
filter	mortorized wheel 5-position 535nmBPF / 595nmBPF / 680nmBPF / ND
view size	10 × 7.5cm / 14 × 10cm / 18 × 13cm / 26 × 20cm 4-position
file type	16bit TIFF / 8bit TIFF/JPEG/BMP
dimension	472mm(W) × 480mm(D) × 802mm(H) , 76 kg
power	AC100-240V 50/60Hz 200W

Normalization method by total protein

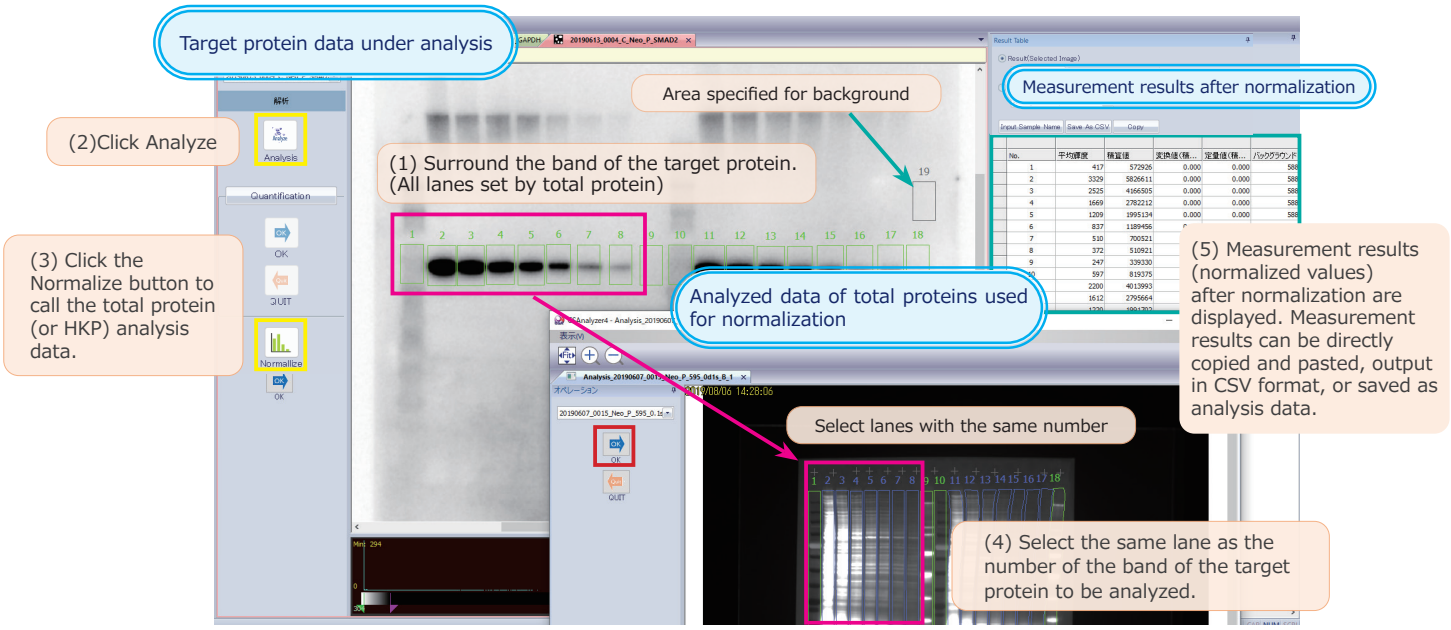
(3) Normalization

After enclosing the target protein band and setting the background, click the “Analyze” button to quantify the signal intensity.



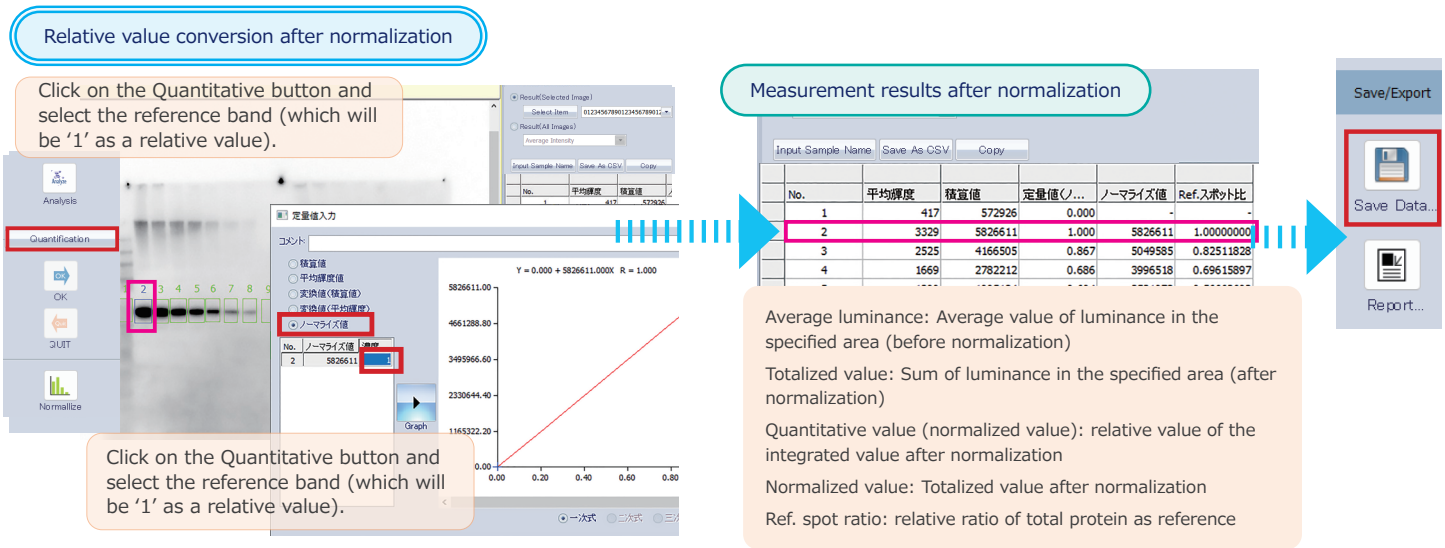
Click the “Normalize” button to call up the analysis data of the reference protein (total protein).

The analysis data of the reference protein is displayed. Click the lane of the total protein with the same number as the band to be normalized to select it and click “OK” . The normalized measurement results can be copied and pasted, exported in CSV format, or saved as analysis data.



(4) Quantitative analysis of normalized values

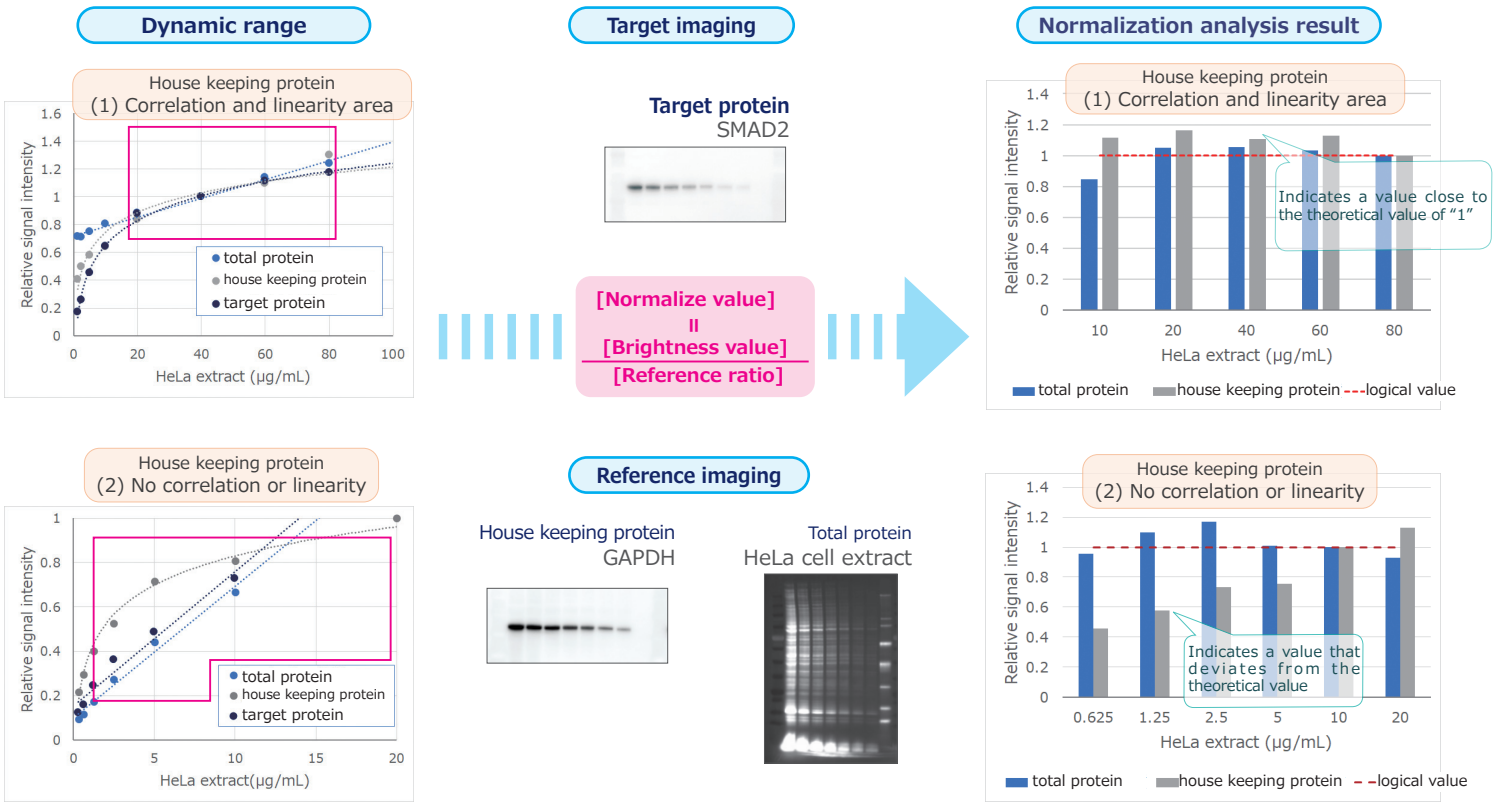
To convert the normalized result as a relative value, click the “Quantitative” button. Click on the reference band to select it and click “OK”. Select “Normalized Value” and enter “1 (or any number)” . The normalized value will be converted as a relative value, which can be copied and pasted, output in CSV format, or saved as analysis data.



7. Normalization Analysis Result

7-1. Comparison of normalized quantification results by total protein and housekeeping protein

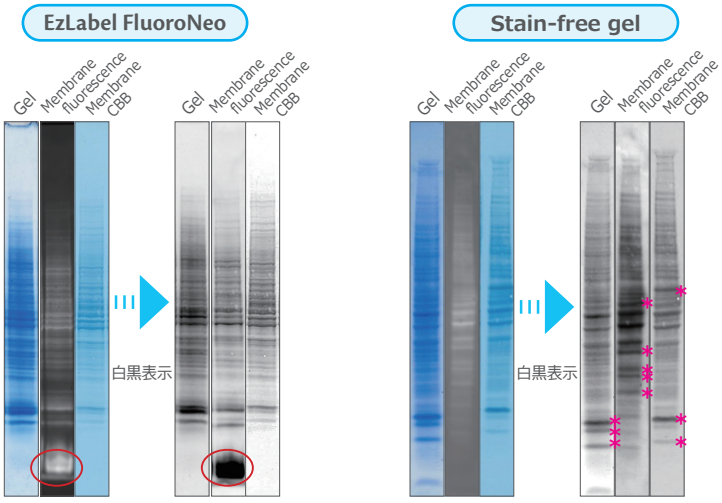
The figure below shows data from Western blotting of EzLabel FluoroNeo-labeled HeLa cell extracts for SMAD2 protein, showing the expression levels of SMAD2 protein in a 1/2 dilution series of samples, normalized by total protein and housekeeping protein. The data are normalized by total protein and housekeeping protein.



If the calibration curves for the target and reference proteins are linear and highly correlated, as shown in (1) above, the normalized results are close to the theoretical value of 1 for both total and housekeeping proteins. However, in general, as shown in (2), housekeeping proteins are abundantly expressed, so the linearity dynamic range of the calibration curve is different from that of the target protein. Therefore, normalized results by housekeeping proteins may deviate from the theoretical value.

7-2. Comparison of sample labeling with EzLabel FluoroNeo and stain-free gel

The bands detected by EzLabel FluoroNeo and stain-free gel are compared in the fluorescence-detected images of the gel, the PVDF membrane after transfer (membrane_fluorescence), and the CBB-stained image (membrane_CBB). To facilitate comparison of the bands, each image is shown in grayscale.



Nonspecific signals derived from foreign substances or amino acids in the sample

Fluorescence detected band pattern is different from CBB staining (*)

The "Membrane_Fluorescence" lane shows the results of detecting bands labeled with fluorescent dyes from EzLabel FluoroNeo or stain-free gels. The reason is that EzLabel FluoroNeo labels the first and second amines, so almost all proteins are equally labeled. On the other hand, the stain-free gel labels tryptophan among the amino acids, so the signal strength depends on the presence or absence and number of tryptophan (right figure). Therefore, the band pattern differs slightly between fluorescence detection and CBB detection. The bands indicated by ** show bands with markedly different detection sensitivity between fluorescence and CBB detection.

Related products

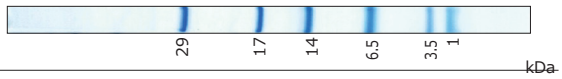
Main precast gel

Code No.	product	gel conc.	分画範囲	検体数	volume		items
2331970	EHR-T520L	5-20%	5～400kDa	14well	max 24μL	mini-size gel、Tris/Glycine buffer 7.5%, 10%, 12.5%, 15%, 10-20%	10/box
2332070	EHR-R520L	5-20%	5～400kDa	18well	max 18μL		
2332260	P-T16.5S	16.5%	1～75kDa	14well	max 24μL	mini-size gel、Tris/Tricine buffer For low molecular weight peptide separation	10/box
2332265	P-R16.5S	16.5%	1～75kDa	18well	max 8μL		
2331605	CHR-520L	5-20%	5～400kDa	15well	max 7.5μL	compakut-seze gel、Tris/Glycine buffer 他 7.5%, 10%, 12.5%, 15%	10/box
2331695	CP16.5S	16.5%	1～75kDa	15well	max 7.5μL	compakut-seze gel、Tris/Tricine buffer For low molecular weight peptide separation	10/box
2332240	M-520L	5-20%	5～400kDa	30well	max 20μL	wide-size gel、Tris/Glycine buffer	6/box

Electrophoresis buffer

Code No.	product	items	
2332310	AE-1410 EzRun	1	10 L of powder Tris-Glycine-SDS High-speed electrophoresis
2332325	AE-1415 EzRun T	1	5L of powder for low molecular protein Tris-Tricine-SDS High-speed electrophoresis
2332326	WSE-7065 EzRun MOPS	1	20 x concentrated solution 250mL/container Tris-MOPS-SDS Further high-speed and wide molecular weight range separation

Molecular weight marker

Code No.	product	items	
2332348	WSE-7025 EzStandard LMW 100μL(20×) for SDS-PAGE	1	
2332346	WSE-7020 EzProtein Ladder 250μL×2 for SDS-PAGE	1	

Sample Preparation Reagents

Code No.	product	items	
2332336	WSE-7420 EzRIPA Lysis kit	1	Extract for animal cells: 100mL with protease inhibitor & phosphatase inhibitor
2332339	WSE-7423 EzBactYeast Crusher	1	Extract for E. coli and yeast: 100mL with protease inhibitor & DNase
2332330	AE-1430 EzApply	1	Sample Preparation Buffer for SDS-PAGE 5 mL × 5 portions
2332333	WSE-7010 EzLabel FluoroNeo	1	Fluorescence labeling for proteins & preparation of electrophoresis samples: 2000 samples with molecular weight markers

Reagents and consumables for blotting

Code No.	product	items	
2322443	WSE-4057 QBlot kit M	1	No filter paper, PVDF membrane, or transfer buffer required Transfer pack of 10 miniature gels
2322451	WSE-4051 Clear Blot P+menbrane	1	20 sheets of pre-cut PVDF film 85 x 90mm 0.2μm for mini size gels (other sizes available)
2322506	WSE-4061 Clear Blot P menbrane (low fluorescence)	1	Pre-cut PVDF film for fluorescence detection for mini size gels 85×90mm 0.2μm 10 sheets (other sizes available)
2332375	WSE-7160 EzStainAQua MEM	1	Wash (500mL), Dye (500mL), Decolorant (500mL), Complete Decolorant (500mL)
2332595	WSE-7210 EzFastBlot HMW	1	5x concentrated solution 500mL High speed semi-dry blotting reagent for high molecular weight proteins
2332590	AE-1465 EzFastBlot	1	10x concentrated solution 500mL High speed semi-dry blotting reagent
2332600	AE-1460 EzBlot	1	Three-component semi-dry blotting reagent Solution A: 475mL, B: 475mLx2, C: 475mL

Other related equipment

Code No.	product	items	
2311130	WSE-3100 PowerStation Ghibli I	1	Touch panel type power supply
2322197	WSE-1165 Rapidas Mini Slab	1	Acrylic electrophoresis chambers for mini size gels and PAGEL series
2321905	AE-6530P Rapidas Mini Slab	1	electrophoresis chambers for mini size gels and PAGEL series
2322210	WSE-1170 Multilane Gel Electrophoresis	1	electrophoresis chambers for wide size gels
2321670	WSE-1150P PageRun Ace	1	electrophoresis apparatus with power supply for mini size gels and PAGEL series
2322252	WSE-1030 CompactPAGE Neo	1	electrophoresis apparatus with power supply for compact size gels and c-PAGEL series
2322466	WSE-4025 HorizeBLOT 2M	1	semidry blotter for mini size gels (x2)
2322476	WSE-4045 HorizeBLOT 4M	1	semidry blotter for mini size gels (x4)
2322496	WSE-4125 PoweredBLOT 2M	1	semidry blotter with power suply for mini size gels (x2)

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