



FastGene®

WESTERN BLOT SYSTEM



PG08 Manual

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1. INTRODUCTION

Thank you for purchasing the FastGene® Western Blot System. The device enables an efficient, electrophoretic wet-transfer of proteins from PAGE gel to membrane.

Before Western Blot transfer, proteins are separated by size via PAGE. PAGE stands for Polyacrylamide Gel Electrophoresis and describes an analytical method in biochemistry for the separation of differently sized protein mixtures in an electric field. During PAGE, proteins migrate through a gel matrix in response to an applied electric field. Smaller proteins travel faster through the gel than larger proteins, leading to a size dependent separation.

The most common form of PAGE is SDS-PAGE. The detergent sodium dodecyl sulfate (SDS) is added for PAGE sample preparation and is also part of the buffer composition. The treatment with the harsh detergent leads to full protein denaturation and unfolding. SDS binds to hydrophobic parts of the unfolded protein and masks the intrinsic charge of the protein with its own negative charge. As a consequence, SDS-protein complexes migration is predominantly dependent on the size of the protein, allowing an estimation of its molecular weight.

Blotting of proteins is a powerful biochemical method for the detection and analysis of proteins. Protein bands that were separated by size after PAGE are transferred and immobilized on a carrier membrane. The firm binding of the proteins to the membrane allows a subsequent protein detection by choosing from a variety of staining or immunological methods.





2. SYSTEM COMPONENTS

2.1 Ordering Information

Following sets / components are available, additionally to the FastGene® Western Blot System (PG08):

Cat. No.	Product	Content
PG08	FastGene® Western Blot System	Complete Western Blot set (for content list see table on next page)
PG09	FastGene® Western Blot components	Western Blot components (for content list see table on next page)
PG05	FastGene® Gel/Blot chamber tank	Large gel electrophoresis buffer tank (1 chamber tank)



2.2 Western Blot System (PG08)

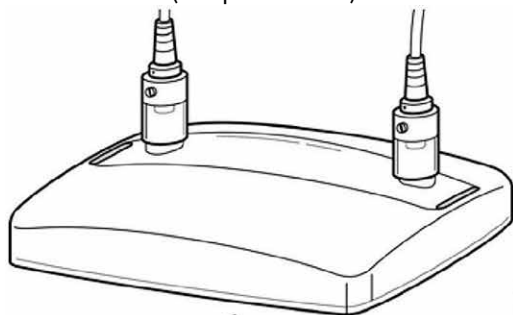
Please check the system and the packing list when you first open the packing case of the FastGene® Western Blot System. If you find there is something wrong with the system or the packaging content, please contact NIPPON Genetics EUROPE or your local distributor.

The complete FastGene® Western Blot System set (PG08) enables to execute the electrophoretic wet-transfer of proteins from gel to membrane. PG08 contains all components needed for Western Blot transfer. If you already possess the FastGene® Gel/Blot chamber tank (PG05) and chamber lid with electrodes, they can be expanded with the FastGene® Western Blot components (PG09)

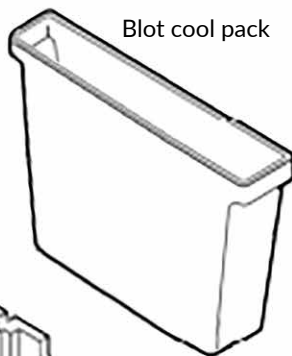
FastGene® Western Blot System (PG08)		Qty
Blot frame with electrodes	PG09	1
Blot connector		2
Blot sponge		5
Blot cool pack		2
FastGene® Gel/Blot chamber lid with electrodes (and power cable)		1
FastGene® Gel/Blot chamber tank (PG05)		1



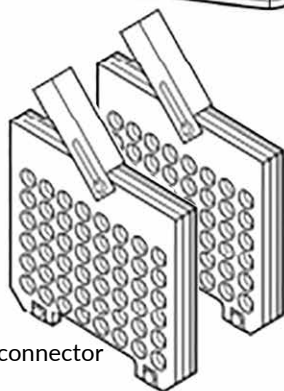
FastGene® Gel/Blot chamber lid
with electrodes (and power cable)



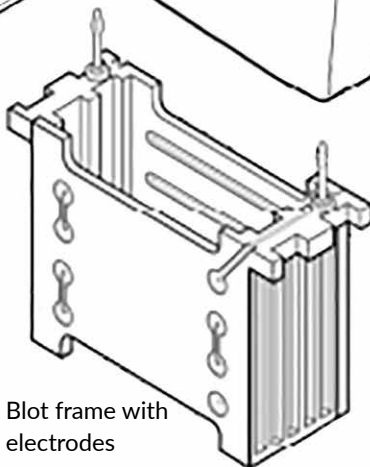
Blot cool pack



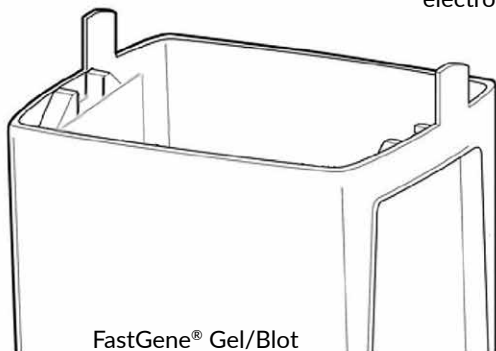
Blot connector



Blot frame with
electrodes



FastGene® Gel/Blot
chamber tank



Blot sponge

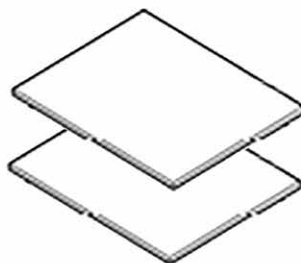


Fig. 1: Western Blot system parts



Description of Western Blot system parts (depicted in Fig. 1):

Blot Frame with Electrodes:

Holds two blot connectors. The blot frame with electrodes is filled with running buffer during the electrophoretic protein transfer run. The lid is connected to the blot frame with electrodes.

Blot connector:

Holds the gel sandwich for the electrophoretic transfer of proteins from PAGE gel to membrane. The gel sandwich consists of two blot sponges, two filter papers, a PAGE gel and membrane (see page 12 for detailed description of the gel sandwich).

Blot sponge:

Two blot sponges are used within the blot connector to allow a tight compression between membrane and PAGE gel and to allow good protein transfer.

Blot cool pack:

The blot cool pack is frozen at -20°C and inserted into the tank before protein transfer. It is used to absorb the generated heat during blotting and to cool down the system.

FastGene® Gel/Blot chamber lid with electrodes (and power cable):

The lid is positioned on top of the blot frame with electrodes and the FastGene® Gel/Blot chamber tank. The power cables (cathode and anode) are connected to the power supply.

FastGene® Gel/Blot chamber tank:

The chamber tank holds the blot frame with electrodes. The chamber is filled with running buffer during the blotting run.

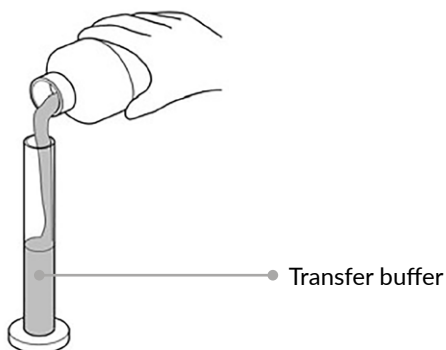


3. SYSTEM OPERATION

Please read the instructions manual carefully and operate the system accordingly.

3.1 Western Blot System Assembly

- 1 Prepare transfer buffer. Refer to Page 19 (4.1) for different buffer recipes. Approximately 200 ml of transfer buffer are needed for equilibration of gels, membranes and other transfer materials and approximately 500 ml are needed to fill the FastGene® Gel/Blot chamber tank. Prepare a total of ~750 ml transfer buffer per run.



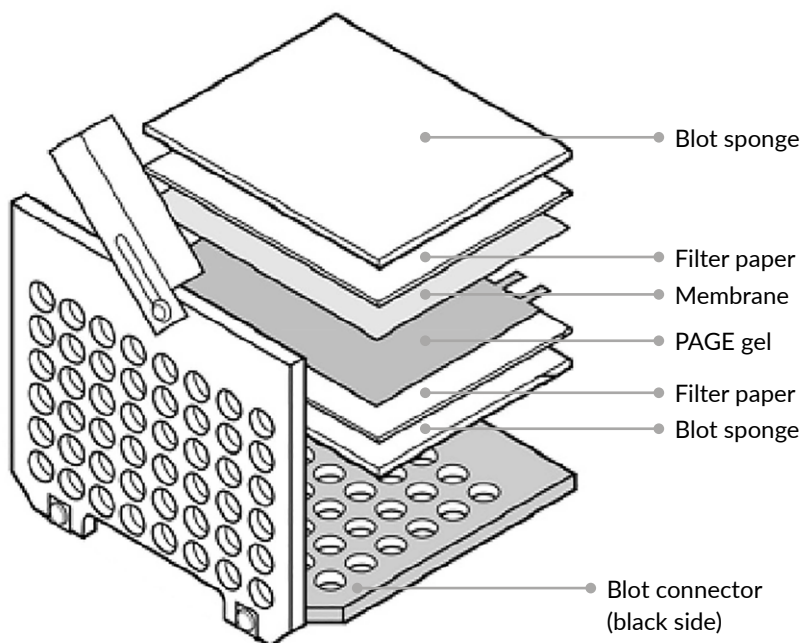
- 2 For setting up the protein transfer sandwich, prepare the following items: western blot transfer membrane, PAGE gel, two filter papers and two blot sponges. Refer to Page 20 for the description of different types of transfer membranes. Cut filter papers and membrane according to PAGE gel size. Prepare a bath with ~200 ml of transfer buffer. Equilibrate filter papers, blot sponges and membranes for ~5 min. If using PVDF membranes, soak them in 100 % methanol for ~1 min before equilibration in transfer buffer. Remove the PAGE gel from the gel cassette, rinse the gel with deionized water and equilibrate in transfer buffer for 5 min.



3

Prepare the protein transfer sandwich:

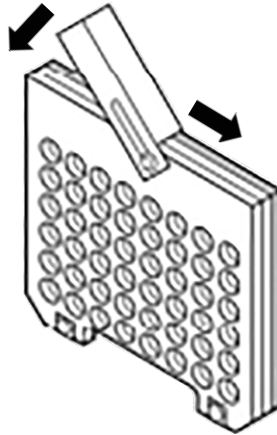
1. Open the blot connector and put it on a clean desk.
2. First, put a wet blot sponge on the black half of the blot connector.
3. Then, put an equilibrated filter paper on top of the blot sponge.
4. Continue with the equilibrated PAGE gel. Make sure that no air bubbles are trapped between the PAGE gel and filter paper. If air bubbles are present, carefully remove them with a wet glass rod or plastic rod.
5. Put the equilibrated membrane on top of the PAGE gel (remove air bubbles) and continue with an equilibrated filter paper (remove air bubbles).
6. Top the protein transfer sandwich with a blot sponge.





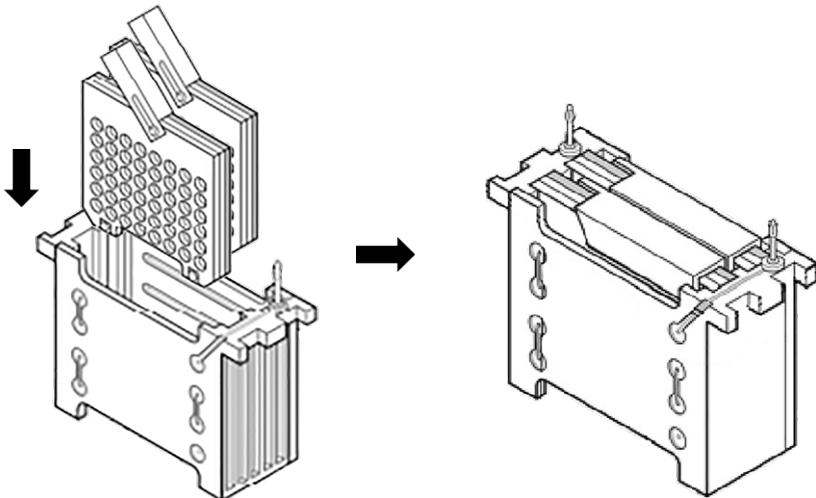
4

Fold down the transparent half of the blot connector on top of the protein transfer sandwich. Make sure not to move any of the transfer components to avoid air trapping. Tighten the blot connector clamp to secure the protein transfer sandwich by folding down the clamp and then sliding it to the middle of the blot connector. Repeat steps one to four, if two western blot transfers are run simultaneously.



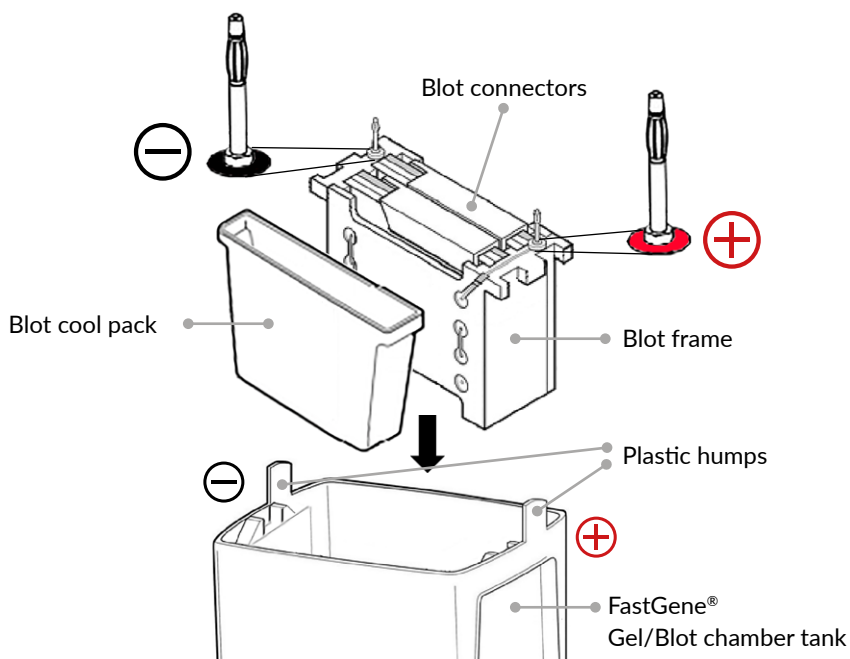
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Slide the blot connector(s) into the blot frame with electrodes with the black side of the blot connector(s) facing towards the black side of the blot frame.





- Put the assembled blot frame together with the cold (-20°C) blot cool pack into the FastGene® Gel/Blot chamber tank. Make sure to align the electrodes of the blot frame with the plastic humps of the FastGene® Gel/Blot chamber tank. Also make sure, that the anode (red) and cathode (black) of the blot frame are aligned with the anode (+) and cathode (-) symbols on the FastGene® Gel/Blot chamber tank.

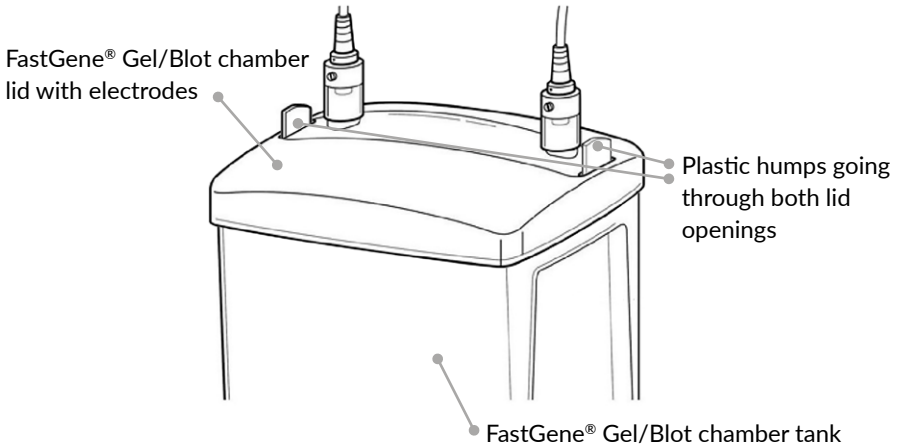


- Fill the FastGene® Gel/Blot chamber tank with transfer buffer up to the indicator mark (Blotting). Put a magnetic stirrer into the tank and place the tank on top of a magnetic stir plate. Start stirring to ensure an evenly distributed temperature and conductivity.



8

Put the FastGene® Gel/Blot chamber lid on top of the Gel/Blot chamber tank. It will only be possible to put the lid on the chamber tank if the blot frame is correctly positioned inside the chamber tank. Make sure that the plastic humps of the Gel/Blot chamber tank go through the two narrow openings on each side of the lid.





3.2 Electrophoretic Transfer

- 1 Connect the electrodes of the lid to an external power supply. Do not attempt to operate the electrophoresis system without the lid.
- 2 Perform protein transfer. The optimal transfer conditions depend on the protein properties such as size, charge, and protein mobility. It also depends on the used transfer buffer, gel thickness and transfer system. The protocol should be individually tested, optimized and adapted. Nevertheless, there are standard conditions to consider as a rough guideline. Using one of the SDS-PAGE gel transfer buffers (Page 19, 4.1), a standard field strength or high field strength transfer can be applied:
 1. Standard field strength transfer is performed with less power input over a longer time period. It offers a good transfer for a broad protein molecular weight range.
 2. High field strength is applied for a shorter amount of time and produces satisfactory results in most cases. However, in some cases it can cause small sized proteins to be transferred through the membrane, or not offer sufficient transfer time for larger proteins.

	SDS-PAGE gel protein transfer	
	Standard field strength	High field strength
Voltage	30 V	100 V
Current	90 mA	350 mA
Time	over night	1 h

The transfer can be performed under constant voltage or constant current:

1. Applying constant voltage results in an increase of power over time, to compensate for the resistance drop due to increased heating. This results in more power and heating increase over time. Constant voltage ensures a more efficient transfer but creates more heat and should be used with a cooling element (blot cool pack).
2. When applying constant current, the decrease of resistance leads to a decrease in voltage. This results in a decrease of protein transfer over time, but also less heat production.



- 3** Turn off the power of the external power supply and disconnect the electrodes, after the protein transfer run is completed. Open the lid, take out the blot frame with the blot connector(s) from the buffer tank and pour out the running buffer. Slide open the clamp from the blot connector and open the protein transfer sandwich. Remove the membrane and continue with a blot detection method of choice.

- 4** Do not reuse the transfer buffer. Discard the transfer buffer and clean the blot connector, blot frame, blot sponges and gel/blot chamber tank with distilled water. Discard used filter papers and PAGE gel.





4. TRANSFER COMPONENTS

4.1 Transfer Buffer Recipes

The FastGene® Gel/Blot chamber tank holds a buffer volume of ~ 500 ml. To achieve best results, only use high quality grade chemicals for buffer preparation.

Methanol can also be substituted for ethanol in most cases, without a noteworthy reduction of transfer efficiency. The protocol using ethanol should be individually tested, optimized and adapted. Do not adjust the pH of the buffers.

The following standard buffers can be used for SDS-PAGE gel protein transfer.

	Buffer	Recipe	
A	25 mM Tris 192 mM glycine 20% (v/v) methanol (optional 0.1 % SDS) pH 8.3	Tris Glycine H ₂ O (distilled) Methanol SDS (optional)	3.03 g 14.4 g 500 ml 200 ml adjust volume to 1 l with H ₂ O (distilled) add 10 ml 10 % SDS to 1 l buffer
B	48 mM Tris 39 mM glycine 20% (v/v) methanol (optional 0.0375 % SDS) pH 9.2	Tris Glycine H ₂ O (distilled) Methanol SDS (optional)	5.82 g 2.93 g 500 ml 200 ml adjust volume to 1 l with H ₂ O (distilled) add 3.75 ml 10 % SDS to 1 l buffer
C	10 mM NaHCO ₃ 3 mM NaCO ₃ 20 % (v/v) methanol pH 9.9	NaHCO ₃ NaCO ₃ H ₂ O (distilled) Methanol	0.84 g 0.318 g 500 ml 200 ml adjust volume to 1 l with H ₂ O (distilled)
D	10 mM CAPS 20% (v/v) methanol pH 11	CAPS Methanol H ₂ O (distilled)	2.21 g 100 ml 500 ml adjust volume to 1 l with H ₂ O (distilled) adjust pH with NaOH if necessary



4.2 Blotting membranes

Different kinds of blotting membranes can be selected for protein transfer, depending on the desired applications. They are commonly available in two pore sizes. Recommended for most analytical protein blotting procedures are 0.45 μm pore size membranes. Membranes with a pore size of 0.2 μm are recommended for smaller proteins with a molecular weight of <15000 kDa, that could pass membranes with larger pores.

4.2.1 Nitrocellulose membranes

Nitrocellulose membranes are widely used for western blotting and protein detection. Colorimetric protein staining can be accomplished by protein specific dyes such as amido black, coomassie blue, ponceau S or colloidal gold. Nitrocellulose membranes are also used in immunological detection methods such as radioimmunoassays, chemiluminescence-immunoassays, fluorescence-immunoassays and enzyme-linked immunoassays. Proteins can bind strongly and almost irreversibly to the membrane with 80- 100 $\mu\text{g}/\text{cm}^2$.

Membrane	Pore Size	Protein binding capacity	Detection Methods	Comments
Nitrocellulose	0.2 μm 0.45 μm	80 - 100 $\mu\text{g}/\text{cm}^2$	Colorimetric Chemiluminescence Chemifluorescence Fluorescence Radioactive	All-purpose protein blotting membrane

4.2.2 PVDF membranes

PVDF (polyvinylidene difluoride) membranes can be used for amino-terminal sequencing, amino acid analysis and various kind of immunoassays. They retain bound protein even in the presence of organic solvents or acidic/basic conditions. PVDF membranes show a better binding efficiency of proteins with presence of SDS in the transfer buffer. They must be soaked with 100% methanol before use.

Membrane	Pore Size	Protein binding capacity	Detection Methods	Comments
PVDF	0.2 μm	150 - 160 $\mu\text{g}/\text{cm}^2$	Colorimetric Chemiluminescence Chemifluorescence Fluorescence Radioactive	High mechanical strength and chemical stability



5. TROUBLE SHOOTING

Electrophoretic transfer:

Problem	Cause	Solution
<ul style="list-style-type: none">Poor protein transfer, weak bands on blot after staining	<ul style="list-style-type: none">Wrong power conditions or transfer time too short	<ul style="list-style-type: none">Increase transfer timeIncrease power / voltage
	<ul style="list-style-type: none">Transfer time too high (proteins transported through membrane)	<ul style="list-style-type: none">Decrease transfer timeReduce power / voltage
	<ul style="list-style-type: none">Wrong buffer composition	<ul style="list-style-type: none">Check buffer compositionUse high quality chemicals
	<ul style="list-style-type: none">Wrong protein transfer direction	<ul style="list-style-type: none">Check assembly of the protein transfer sandwich in the blot connectorCheck the facing direction of the blot connector(s) in the blot frame
	<ul style="list-style-type: none">Wrong protein charge-to-mass ratio	<ul style="list-style-type: none">Use a different transfer buffer to increase protein mobilityUse buffer with two pH units higher or lower than the isoelectric point of protein for optimal transfer efficiency
	<ul style="list-style-type: none">Protein precipitation in gel	<ul style="list-style-type: none">Use transfer buffer recipe with SDSReduce alcohol concentration in transfer buffer
	<ul style="list-style-type: none">Gel percentage too high	<ul style="list-style-type: none">Use PAGE gel with lower acrylamide percentage



Electrophoretic transfer:

Problem	Cause	Solution
<ul style="list-style-type: none">Regions or spots of poor protein binding on blot, bands missingwhite spots on membrane	<ul style="list-style-type: none">membrane not uniformly wet	<ul style="list-style-type: none">make sure all components are completely wet and soaked in transfer buffermake sure PVDF membrane is soaked in methanol before soaking it with transfer buffer
	<ul style="list-style-type: none">not enough buffer in gel/blot buffer tank	<ul style="list-style-type: none">fill buffer tank completely until the indicator mark is reached
	<ul style="list-style-type: none">poor contact between gel and membrane	<ul style="list-style-type: none">make sure no air bubbles are trapped between gel and membraneuse thicker filter papersreplace blot sponges
<ul style="list-style-type: none">Poor binding to the membrane	<ul style="list-style-type: none">methanol concentration in transfer buffer too high, reduces protein elution from PAGE gel	<ul style="list-style-type: none">reduce amount of methanol in transfer buffer, keep methanol concentration at 20 % for optimal transfer and binding
	<ul style="list-style-type: none">SDS concentration in transfer buffer too high, reduces binding efficiency of proteins	<ul style="list-style-type: none">Reduce SDS concentration or remove SDS completely from transfer buffer
	<ul style="list-style-type: none">Smaller proteins (<15 kDa) passed through membrane	<ul style="list-style-type: none">use PVDF membrane or nitrocellulose membrane with smaller pore sizeDecrease voltage when using high field strengthPlace second membrane in the gel sandwich to detect proteins that are passed through the first membrane



6. WARRANTY

NIPPON Genetics EUROPE provides a product warranty to the purchaser against defects and/or incorrect material processing **for one year after the shipping date**. This warranty excludes damages resulting from shipping, misuse, carelessness, or neglect. NIPPON Genetics EUROPE's liability under the warranty is limited to the receipt of adequate evidence by the customer that the defect falls under the warranty conditions. All claims under this warranty must be submitted to NIPPON Genetics EUROPE within one year of delivery of the product to the customer.

7. CONTACT INFORMATION

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