

APPLICATION NOTE

BlueBlock PF (10x) for Blotting and ELISA

Cat. no. 42591

Vers 0520

To get a good signal-to-noise ratio, it is important to block non-specific antibody binding sites either on the transfer membrane or the ELISA plate.

Protein based blocking solutions like skim milk, BSA or casein solutions may not only block unspecific binding sites, but mask as well specific binding sites.

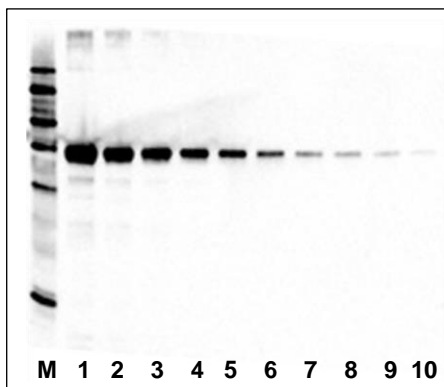
With BlueBlock PF (protein-free, polymer-based), the specific binding sites remain accessible while non-specific reactions are suppressed, thus leading to an increase in signal intensity.

SERVA BlueBlock PF is a 10-fold concentrated, protein-free, polymer-based blocking reagent which can also be used for antibody-dilution. It does not contain any sodium azide (NaN₃).

Features and Benefits

- Blocking of unspecific binding sites
- Protein-free, polymer-based – Does not contain any phospho-proteins like casein-skim milk-based blocking reagents - Suitable for phosphor-protein analysis
- Specific binding sites are retained - increased signal strength and sensitivity (figure 1)
- Short blocking times – 5 min (figure 2)
- *All-in-One*-Protocol – Blocking and antibody incubation in one step (figure 3)
- Compatible with common Western blot detection systems, e.g. colorimetric, fluorescence and chemiluminescence (figure 4)
- Also suitable for ELISA applications

Figure 1: Signal strength and sensitivity



Marker (M): SERVA VisiBlot Standard I (SERVA cat. no. 39260)

Sample (1 - 10): human Transferrin (SERVA cat. no. 36756)

Sample volume: 5 µl/lane

Lane	1	2	3	4	5	6	7	8	9	10
[ng/lane]	500	250	125	62.5	31.3	15.6	7.8	3.9	1.8	0.9

Blocking: 30 min 1x SERVA BlueBlock

1st antibody: Rabbit Anti-Human Transferrin (DAKO A0061)

1:10,000 in 1x SERVA BlueBlock

2nd antibody: Anti-Rabbit-IgG –HRP (Sigma A0545)

1:10,000 in 1x SERVA BlueBlock

Chemiluminescence detection

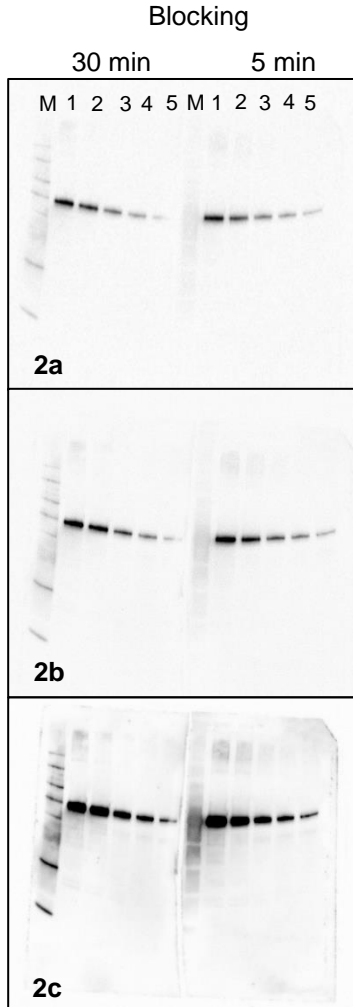
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Figure 2: Blocking Time and Efficiency



Marker (M): SERVA VisiBlot Standard I (SERVA cat. no. 39260)
Sample (1 - 10): Human Transferrin (SERVA cat. no. 36756)
Sample volume: 5 µl/lane

Lane	1	2	3	4	5
[ng/lane]	40	20	10	5	2,5

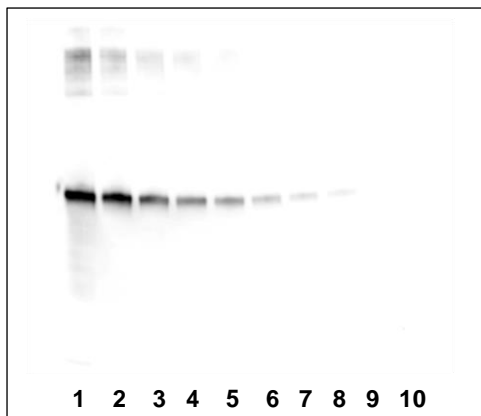
1st antibody: Rabbit Anti-Human Transferrin (DAKO A0061);
1:10,000 in 1x SERVA BlueBlock

2nd antibody: Anti-Rabbit-IgG –HRP 1:10,000 (Sigma A0545)
in 1x SERVA BlueBlock

Chemiluminescence detection (SERVA Musketeer)

Exposition time: 2a – 1 s; 2b – 10 s; 2c – 60 s

Figure 3: All-in-One-Protocol



Marker (M): SERVA VisiBlot Standard I (SERVA cat. no. 39260)
Sample (1 - 10): human Transferrin (SERVA cat. no. 36756)
Sample volume: 5 µl/lane

Lane	1	2	3	4	5	6	7	8	9	10
[ng/lane]	500	250	125	62.5	31.3	15.6	7.8	3.9	1.8	0.9

1st antibody (Ab): Rabbit Anti-Human Transferrin (DAKO A0061)

2nd antibody (Ab): Anti-Rabbit-IgG –HRP (Sigma A0545)

Antibody solution: 2 µl 1st Ab + 2 µl 2nd Ab in 20 ml 1x SERVA BlueBlock

Blocking & Ab incubation:

Blot membrane in 20 ml antibody solution 60 min at RT

Chemilumineszenz detection

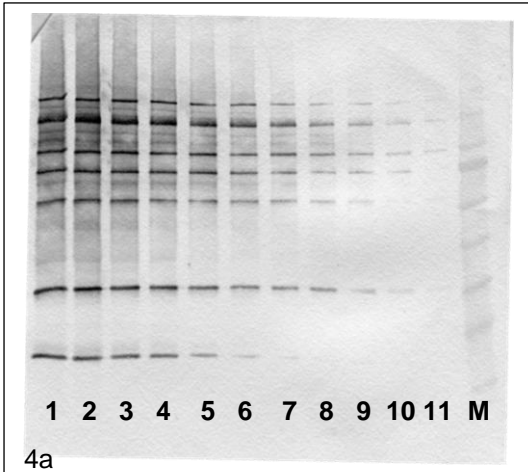
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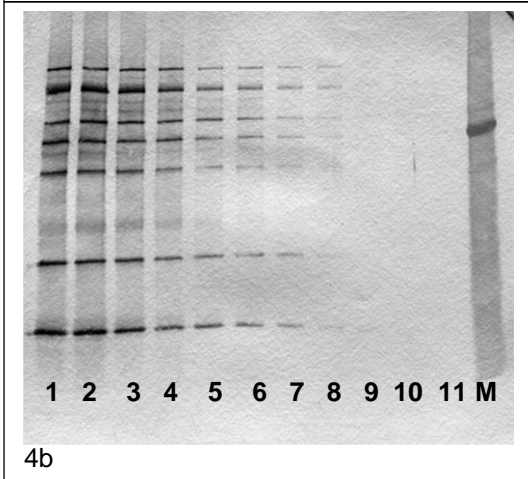
Figure 4: Detection with Chromogenic Substrates



Marker (M): SERVA Triple Color Standard II (SERVA cat. no. 39257)
 Sample (1 - 11): SERVA VisiBlot Standard I (SERVA cat. no. 39260)
 Sample volume: 5 µl/lane

Lane	1	2	3	4	5	6	7	8	9	10	11
Dilution factor	2	2	4	8	16	32	64	128	256	512	1024

Detection:
 4a - Anti-Goat-IgG-AP (1: 5,000) - BCIP/NBT
 4b - Anti-Goat-IgG-HRP (1:5,000) - TMB



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Recommended Standard Protocol for Western Blotting and ELISA

	Western Blot	ELISA
Dilution	Dilute the 10x concentrate with dist. water to get a 1x solution, e.g. 10 ml BlueBlock PF + 90 ml dH ₂ O Mix well	
Blocking	5 min (optimal: 30 min)	1 h
1st Ab Incubation	Incubation with 1x solution at RT on a shaker Optional: At + 2 °C to + 8 °C overnight	
Wash	Dilute the antibody (Ab) with 1x BlueBlock PF 1 h incubation at RT on a shaker	
2nd Ab Incubation	3 x 5 min with PBS-T or TBS-T	
Wash	Dilute the Ab with 1x BlueBlock PF 1 h incubation at RT on a shaker	
Detection	3 x 5 min with PBS-T or TBS-T	
	Using the appropriate substrate for detection	

Recommended All-in-one-Protocol for Western Blots (Mini Gel Format)

Dilution	Dilute the 10x concentrate with dist. water to get a 1x solution, e.g. 10 ml BlueBlock PF + 90 ml dH ₂ O Mix well
Antibody Solution	1x BlueBlock solution + 1 st Antibody + 2 nd Antibody Dilute 1 st and 2 nd antibody as recommended e.g. 1:2,000 (1 st Ab) and 1:5,000 (2 nd Ab)

IMPORTANT: Incubate the antibody solution 5 min prior use.

Membran Wash	5 min with PBS-T or TBS-T
Blocking & Ab Incubation	Add 20 ml antibody solution to the Blot membrane 1 - 2 h incubation at RT on a shaker
Wash	3 x 5 min with PBS-T or TBS-T
Detection	Using the appropriate substrate for detection

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Ordering Information

Product	Cat. no.	Size
Buffers		
BlueBlock PF (10x) for Blotting & ELISA	42591.01	250 ml
	42591.02	1 L
PBS-T Buffer (10x) , sterile	42597.01	1 L
TBS-T Buffer (10x) , sterile	42598.01	1 L
Substrates for Horse Radish Peroxidase (HRP)		
SERVA <i>Light</i> Polaris CL HRP WB Substrate Kit	42584.01	100 ml
	42584.02	250 ml
	42584.03	500 ml
SERVA <i>Light</i> Eos CL HRP WB Substrate Kit	42585.01	50 ml
	42585.02	250 ml
	42585.03	500 ml
SERVA <i>Light</i> Eos Ultra CL HRP WB Substrate Kit	42586.01	20 ml
	42586.02	100 ml
SERVA <i>Light</i> Helios CL HRP WB Substrate Kit	42587.01	20 ml
	42587.02	100 ml
	42587.03	200 ml
SERVA <i>Light</i> Vega CL HRP WB Substrate Kit	42588.01	50 ml
	42588.02	250 ml
	42588.03	500 ml
SERVAColor TMB Blot Solution	37071.01	100 ml
	37071.02	250 ml
TMB ready-to-use substrate for blotting	37070.01	100 ml
Substrates for Alkaline Phosphatase (AP)		
SERVAColor BCIP/NBT Blot Solution	15245.01	250 ml
BCIP/NBT ready-to-use substrate for blotting	15246.01	100 ml

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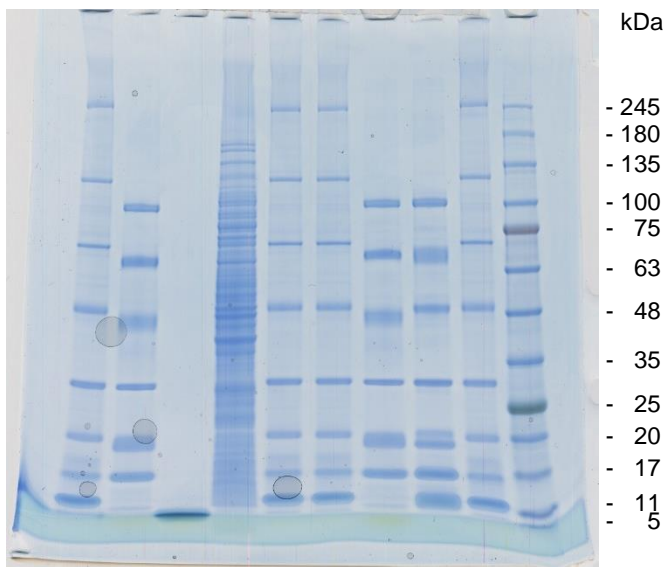
IF TIME MATTERS

Short Blocking and Incubation Times are Nothing Without Fast Electrophoresis and Blotting Procedures - Please find below the appropriate products.



1. Electrophoresis: SERVA Gel™ Neutral HSE

- HSE = *High Speed Electrophoresis*
- Running conditions with the Laemmli buffer system: 400 V, **20 min**



Ordering Information:

Product	Sample wells	Cat. no.
SERVA Gel™ Neutral HSE	15	43249.01
	12	43245.01
	10	43246.01
	2D	43247.01

2. Transfer: SERVA Xpress Blotting Kits

- Ready-to-use
- Even transfer of high and low MW proteins within **15 min**
- Blotting Fleece instead of blotting paper for efficient, undisturbed transfer in a short time



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Ordering Information

Product	Kit Components (Blot format 80 x 85 mm)	Cat. no.
Xpress Blotting Kit for Westen Blotting	250 ml 10x SERVA Xpress Blotting Buffer 20x Blotting fleece 10x Connection Paper	42662.01
Xpress NC Blotting Kit for Westen Blotting	250 ml 10x SERVA Xpress Blotting Buffer 20x Blotting fleece 10x Connection Paper 10x Nitrocellulose Blot membrane (Pore size: 0.2 µm)	42663.01
Xpress PVDF Blotting Kit for Westen Blotting	250 ml 10x SERVA Xpress Blotting Buffer 20x Blotting fleece 10x Connection Paper 10x PVDF Blot membrane (Pore size: 0.2 µm)	42664.01