SpeedBlot (Mouse)

Order # PR-SB02-01

Mo Bi Tec
MOLECULAR BIOTECHNOLOGY

Contents

For research use only!

1. Description

SpeedBlot (Mouse) is a ready-to-use reagent for the detection of primary mouse antibodies in blot assays. This product contains a polyclonal antibody reactive to mouse IgG (Fc) which is linked to spherical gold nanoparticles. After addition of primary antibody the solution can be used for instant detection of immobilized target protein. Standard applications are shortened versions of Western or dot blots.

Read-out:	Visually or image scan
Target molecule:	Mouse IgG (Fc)
Applications:	Western blot from SDS PAGE Dot blot from purification fractions Dot blot for quantification Slot blot from expression studies

2. Kit Content

Product Name	SpeedBlot (Mouse)
Order#	PR-SB02-01
Kit Contents	100 ml (4 x 25 ml) SpeedBlot (Mouse), user manual
Typical Working Volumes	Typically, 10 ml are being used per blot.
Storage	SpeedBlot (Mouse) should be stored at room temperature (RT), ideally 18 °C to 25 °C.
Stability	Originally sealed, the shelf life is ≥ 6 months. Upon opening the reagent should be used up within 3 months.
Form / Appearance	Liquid / Pink (absorption maximum at around 530 ± 6 nm)
Buffer Composition	PBS supplemented with 4% sucrose, 3% BSA, 250 µM EDTA, 0.08% sodium azide
Clonality	Polyclonal
Detection Limit	Approx. 10 fmol target protein per spot
Specificity	Cross reactivity with other immunoglobulins and light chains is less than 0.1%

3. Terms & Conditions

Product Usage: For *In Vitro* Laboratory Research Use Only. NOT to be administered to humans or used for medical diagnosis.

Limited Product Warranty: We offer a LIMITED PRODUCT WARRANTY to our customers. This warranty is limited to 6 months from date of shipment and limits our liability to replacement of this product. No other warranties of any kind, express or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by MoBiTec GmbH. We shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

4. Quality Control

The reagent is manufactured under strict quality control ensuring consistent high product standard.

5. Safety Instructions

The reagent contains 0.08% w/v sodium azide as a preservative.

Nanoparticles influence on the body is not fully understood, thus exposure should be prohibited. Especially vapor and aerosol generation should be prevented avoiding inhalation. Direct skin and mucosal contract should be circumvented.
Accidental spillage should be absorbed and disposed following local regulation. When working with the reagent, wear standard lab safety equipment, like gloves, lab coat, and safety goggles.

6. SpeedBlot (Mouse) Protocol

Short Protocol:

- Rinse the membrane providing the immobilized protein briefly with tap water to remove denaturing detergent.
- Place the membrane into a clean tray of suitable size. Blocking is not necessary.
- Cover the membrane with 10 ml SpeedBlot (Mouse) reagent.
- Add mouse-raised primary antibody, e.g., add 150 µl of a 30 µg/ml antibody solution (≙ 30 pmol or 4.5 µg). You may use higher or lower volumes of SpeedBlot (Mouse) reagent. In this case we recommend to adjust the amount of primary antibody accordingly to ensure a good performance of the product (e.g.: add 225 µl of 30 µg/ml antibody solution to 15 ml of reagent).

- Incubate the membrane on a shaker until the desired bands are visible (usually for 30-90 min). Soft shaking might reduce the detection time. If you don't have a shaker ensure a sufficient degree of mixing after addition of the primary antibody.
- Rinse the membrane swiftly with tap water and/or allow it to dry.
- Take a picture or scan the membrane for electronic documentation. The dried membrane might be stored without any further treatment.

Detailed Protocol:

Western Blotting:
Electro blotting is performed as usual, e.g., transfer protein from SDS-PAGE onto nitrocellulose (NC), polyvinylidene fluoride (PVDF), or nylon membrane in semi-dry blotting procedure.

Dot or Slot Blotting:
Alternatively, fractions from cell extracts or column purification are spotted onto a suitable membrane.

- Immediately after electro transfer rinse the blotting membrane briefly with tap water to remove remaining detergent. If no detergent was used, e.g., in slot or dot blotting applications, this step can be omitted. There is no blocking and additional washing step required.
- Place the membrane into a clean plate or tray of suitable size. Pour 10 ml SpeedBlot (Mouse) reagent onto the membrane covering it completely.
- Add mouse-raised primary antibody suitable to detect your protein of interest. For example, add 150 µl of a 30 µg/ml antibody solution (≙ 30 pmol or 4.5 µg). If you want to use a higher or lower SpeedBlot (Mouse) reagent volume, please adjust the amount of antibody accordingly. We recommend to gently mix the reagent with the antibody if you don't use a shaker in the next step.
- Incubate the membrane at RT until the desired bands are visible (usually for 30-90 min). Soft shaking might reduce the detection time. The target protein becomes visible due to antibody-induced gold nanoparticle accumulation. When the staining is sufficiently intense, proceed with the next step.
- Remove excess reagent and rinse the membrane swiftly with tap water and/or allow it to dry. Background stain will disappear upon drying.
- There is no additional equipment required to detect the stained proteins on the membrane. The specific pink/red signal is visible by the naked eye and may be documented using a camera or a scanner.

- To store the membrane it might be kept dried and covered with filter paper.

The intensity of the band is proportional to the amount of protein immobilized. Thus quantification can be achieved by co-detection of a standard protein dilution spotted next to the sample.

7. Troubleshooting

Problem	Possible cause	Suggestion
No signal	Remaining detergent has denatured the reagents antibody, or the reagent faded because it has been contaminated or frozen.	Rinse the membrane and the tray with tab water and pour fresh reagent into the tray; add the required amount of mouse-raised primary antibody and repeat the staining procedure.
	Added antibody does not bind to the reagent or target.	Rinse the membrane and the tray with tab water and pour fresh reagent into the tray, add a new batch or alternative clone of mouse-raised primary antibody and repeat the staining procedure.
	No or not sufficient target protein present on the membrane	Increase the incubation time, which supports the detection limit. If this does not help, blot again using increased protein amount, or fresh protein, as degradation might affect the antibody binding efficiency. Use a positive control. Check the sequence for frame shifts/stop codons.
Non-specific bands	Long incubation may result in detection of aggregated/degraded protein or cross reactive proteins.	Use fresh/less protein and/or shorten incubation time. Check the protein's DNA sequence for frame shifts or stop codons. Mass spec analysis of the sample may provide protein sequence information.
	The primary antibody is not specific.	Blot again, use a new batch or alternative clone of mouse-raised primary antibody and repeat the staining procedure.
	Contamination with other primary antibodies has occurred.	Blot again, wash the tray with tab water and pour fresh reagent into the tray, add a new batch or alternative clone of mouse-raised primary antibody, and repeat the staining procedure.

8. Order Information, Shipping, and Storage

Order#	Product	Amount
PR-SB02-01	SpeedBlot (Mouse)	100 ml
shipped at RT; store at RT		

9. Related Products

Order#	Product	Amount
PR-SB01-01	SpeedBlot (His)	30 ml
PB01	Perfect-Block™ Blocking Reagent (gelatine-based)	100 g
MOBI05	MobiFairy Protein Stabilizer	2 ml
PR-MAG00041-01	M-Beads Magnetic silica beads S-C8, 1.2 µm	2 ml

10. Contact and Support

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