Protocol Booklet

Product Code(s)	HB13797, HB15002, HB18012
Product Name(s)	SuperBlot™ Rapid Single-Step GAPDH Loading Control SuperBlot™ Rapid Single-Step β-Tubulin Loading Control SuperBlot™ Rapid Single-Step β-Actin Loading Control
Purpose	Rapid detection and visualisation of loading controls in Western Blots

Please note: This product is for RESEARCH USE ONLY and is not intended for therapeutic or diagnostic use. Not for human or veterinary use

Contents

Product Overview

SuperBlot™ Rapid Single-Step Loading Control kits are a novel single-step loading control solution for Western Blot that enables the rapid blocking and staining of membranes for loading control proteins in a single stage:

- Simultaneous blocking, primary and secondary antibody incubation
- Works in as little as 15 minutes.
- Saves hours compared to conventional methods.
- Acts as a signal enhancer to increase sensitivity.
- Animal product free (recombinant antibodies).

Components & Storage

This kit contains two components that need recombining before use to create the active reagent:

SKU	Component	Quantity	Storage Temperature
HB15500 HB19910 HB14400	SuperBlot™ Rapid Single-Step Loading Control - Antibody cocktail	1 vial	-20°C
HB18549	SuperBlot™ Rapid Single-Step Blocking Solution with Preservative	100ml	RT

Please note: Items are shipped at room temperature, transfer the antibody cocktail to -20°C upon delivery.

To combine the reagents to create the SuperBlot™ Rapid Single-Step Loading Control:

1. Add around 1ml of SuperBlot™ Rapid Single-Step Blocking Solution with Preservative to the SuperBlot™ Rapid Single-Step Loading Control - Antibody cocktail.
2. Vortex thoroughly and then briefly centrifuge to ensure all liquid is at the bottom of the vial.
3. Transfer the contents of the vial into the remaining SuperBlot™ Rapid Single-Step Blocking Solution with Preservative and mix thoroughly.

Please note: Store the combined reagent at 4°C for up to 3 months. If desired, smaller amounts of the final buffer can be made up at a time and the individual components snap frozen and stored at -20°C.

Protocol

1. Run SDS-PAGE gel and transfer to PVDF membrane following standard protocols (please see our Western Blot protocol)
2. Following transfer briefly rinse the membrane with PBST or TBST to remove excess transfer buffer then add around 10-20ml of SuperBlot™ Rapid Single-Step Loading Control.
3. Incubate the membrane for 60 minutes in SuperBlot™ Rapid Single-Step Loading Control for optimal results.
    a. The signal intensity will increase with longer duration incubations. While bands can be seen after as little as 15 minutes of incubation, a one-hour incubation is around the optimal balance between signal intensity and time saving.
    b. It is not recommended to incubate for longer than 4 hours due to the risk of high background staining.
4. Pour off SuperBlot™ Rapid Single-Step Loading Control (this can be stored at 4°C and reused multiple times).
5. Wash blot 3 times briefly then 3 x 5 minutes with PBST (HB8088) or TBST (HB6971).
6. Develop with a standard ECL substrate such as SuperBlot ECL Western Blotting Substrate Kit (High sensitivity).
7. Image the blot using an appropriate imaging system for chemiluminescence.

Guidelines, precautions, troubleshooting

Please follow the below table to resolve any problems encountered when using this kit. For any problems not listed or for any further advice please contact our technical support team at technicalhelp@hellobio.com.

Problem	Potential Cause
Bands faint / weak	Insufficient incubation time. Try incubating longer (up to 2 hours) for optimal results.
	Low sample protein concentration. Try to load samples containing at least 10µg of total protein for optimal results.
High background signal	Excessive incubation time. Try incubating for a shorter amount of time, 1 hour is around optimal.
	Insufficient washing. Try increasing the number and length of washes. Around 3 fast and 3 slow washes is optimal.
Inconsistent band intensity	Inconsistent loading of samples. Try to ensure each lane has the same amount of total protein in it (around 10-20µg per lane).

Observe safe laboratory practice and consult the safety datasheet. Please see the datasheet on our website for general guidelines, precautions, limitations on the use of the assay kit.

Contact

For customers in the UK, Europe and Rest of the World

Customer Care	customercare@hellobio.com
Technical support	technicalhelp@hellobio.com
By telephone:	+44(0)117 318 0505
By fax:	+44(0)117 981 1601
Opening hours:	8.30 am - 5.00 pm GMT weekdays

For customers in the USA, Canada and South America

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