

EveryBlot Blocking Buffer for Sandwich ELISA Protocol

Introduction

EveryBlot Blocking Buffer is a proven, effective western blotting buffer that provides maximum sensitivity with just 5 minutes of incubation. This versatile blocking buffer can be used in enzyme-linked immunosorbent assay (ELISA) applications. Using the EveryBlot Blocking Buffer in ELISA, users can now eliminate the typical 1 hr blocking incubation step for a more streamlined workflow. EveryBlot Blocking Buffer requires no incubation time without sacrificing the high sensitivity of ELISA assays. With its proprietary blend of blocking agents, EveryBlot Blocking Buffer has been meticulously formulated to effectively minimize background noise while maximizing signal intensity, ensuring unparalleled sensitivity and specificity to achieve reproducible and accurate results with ease.

Materials

- Corning 96-Well Clear Flat Bottom Polystyrene High Bind Microplate (Corning Incorporated, catalog #9018)
- Incubator or plate warmer with 37°C setting
- Pipets and tips
- 5x ELISA Coating Buffer (Bio-Rad Laboratories, Inc., catalog #BUF030)
 - As an alternative coating buffer, use 1.5 g anhydrous Na_2CO_3 plus 2.93 g anhydrous NaHCO_3 in 1 L distilled water, pH 9.6
- EveryBlot Blocking Buffer, 500 ml (Bio-Rad, #12010020)
- 10x ELISA Wash Buffer (Bio-Rad, #BUF031)
 - As an alternative wash buffer, use phosphate buffered saline containing 0.05% v/v Tween-20
- Streptavidin (Bio-Rad, #8429-9004)
- HRP Enzyme Substrate (TMB) (Bio-Rad, #1662402) or TMB Core+ (Bio-Rad, #BUF062), for use with HRP-conjugated antibodies
 - Stop solution for TMB and TMB Core+: 0.2 M H_2SO_4
- pNPP (Bio-Rad, #BUF044), for use with alkaline phosphatase-conjugated antibodies
 - Stop solution for p-nitrophenyl phosphate (pNPP): 1 M NaOH
- ELISA microplate reader

Bio-Rad Protocol for Sandwich ELISA with Streptavidin-Biotin Detection

1. Coat microtiter plate wells with 100 μl of the appropriate coating antibody at 1–10 $\mu\text{g}/\text{ml}$ in coating buffer. Cover the plate and incubate overnight at 4°C. Wash the plate three times in wash buffer.
2. Add 150 μl of EveryBlot Blocking Buffer to each well. Remove the buffer immediately by aspiration or by inverting the plate. Repeat this process two additional times.
3. Wash three times in wash buffer.
4. Add 100 μl of suitably diluted samples to the relevant wells. Ensure that appropriately diluted standards are included (dilute samples and standards in wash buffer). Samples or standards should preferably be run in triplicate. Incubate for 90 min at 37°C or overnight at 4°C. Wash three times in wash buffer.
5. Add 100 μl of biotin-conjugated detection antibody (appropriately diluted in wash buffer) to each well. Incubate for 1 hr at 37°C. Wash three times in wash buffer.
6. Add 100 μl of enzyme-conjugated streptavidin (appropriately diluted in wash buffer) to each well. Incubate for 1 hr at 37°C. Wash three times in wash buffer.
7. Add 100 μl of the appropriate substrate solution to each well. Incubate at room temperature (in the dark if required) for 30 min, or until desired color change is attained.
8. Read absorbance values immediately at the appropriate wavelength or add 50 μl of stop solution. Gently tap plate to ensure thorough mixing. Measure absorbance within 30 minutes.

Visit [bio-rad.com/EveryBlot](https://www.bio-rad.com/EveryBlot) for more information.

BIO-RAD is a trademark of Bio-Rad Laboratories, Inc. All trademarks used herein are the property of their respective owner. © 2024 Bio-Rad Laboratories, Inc.



**Bio-Rad
Laboratories, Inc.**

Life Science
Group

Website [bio-rad.com](https://www.bio-rad.com) **USA** 1 800 424 6723 **Australia** 61 2 9914 2800 **Austria** 00 800 00 24 67 23 **Belgium** 00 800 00 24 67 23 **Brazil** 4003 0399
Canada 1 905 364 3435 **China** 86 21 6169 8500 **Czech Republic** 00 800 00 24 67 23 **Denmark** 00 800 00 24 67 23 **Finland** 00 800 00 24 67 23
France 00 800 00 24 67 23 **Germany** 00 800 00 24 67 23 **Hong Kong** 852 2789 3300 **Hungary** 00 800 00 24 67 23 **India** 91 124 4029300 **Israel** 0 3 9636050
Italy 00 800 00 24 67 23 **Japan** 81 3 6361 7000 **Korea** 82 080 007 7373 **Luxembourg** 00 800 00 24 67 23 **Mexico** 52 555 488 7670
The Netherlands 00 800 00 24 67 23 **New Zealand** 64 9 415 2280 **Norway** 00 800 00 24 67 23 **Poland** 00 800 00 24 67 23 **Portugal** 00 800 00 24 67 23
Russian Federation 00 800 00 24 67 23 **Singapore** 65 6415 3188 **South Africa** 00 800 00 24 67 23 **Spain** 00 800 00 24 67 23 **Sweden** 00 800 00 24 67 23
Switzerland 00 800 00 24 67 23 **Taiwan** 886 2 2578 7189 **Thailand** 66 2 651 8311 **United Arab Emirates** 36 1 459 6150 **United Kingdom** 00 800 00 24 67 23

