# **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 enzymelinked 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**

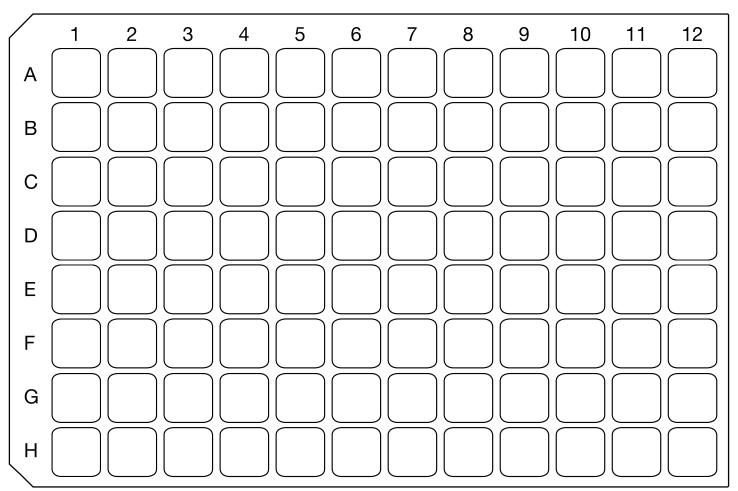
- Corning 96-Well Clear Flat Bottom Polystyrene High Bind Microplate (Corning Incorporated, catalog #9018)
- Incubator or plate warmer with 37°C setting
- Pipets and tips
- 5x ELISA Coating Buffer (Bio-Rad Laboratories, Inc., catalog #BUF030)
  - As an alternative coating buffer, use 1.5 g anhydrous  $Na_2CO_3$  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 HRPconjugated antibodies
  - Stop solution for TMB and TMB Core+: 0.2 M H<sub>2</sub>SO<sub>4</sub>
- 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**

- Coat microtiter plate wells with 100 μl of the appropriate coating antibody at 1–10 μg/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.
- 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.



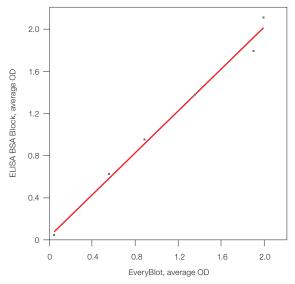
# **Microplate Layout**



# **Comparison of Blocking Buffers**

_	Absorbance Values, OD			
Antigen Concentration, pg	ELISA BSA Block		EveryBlot Blocking Buffer	
400	2.137	2.09	2.016	1.969
200	1.827	1.765	1.804	1.993
100	1.458	1.306	1.34	1.37
50	1.002	0.902	0.895	0.879
25	0.662	0.588	0.558	0.553
0	0.044	0.044	0.044	0.047
_	■ 150 µl/well ■ Incubation time: 1 hr		■ 150 µl/well ■ Incubation time: 0 min	

## EveryBlot Blocking Buffer vs. ELISA BSA Block



**Blocking buffer comparison.** EveryBlot Blocking Buffer and standard ELISA BSA Block (Bio-Rad, #BUF032) showed comparable blocking results in ELISA with a good correlation coefficient (r = 0.99, n = 2 replicate plates). Fit line (—). BSA, bovine serum albumin.

Visit bio-rad.com/EveryBlot for more information.

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