EveryBlot Blocking Buffer for Indirect 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.

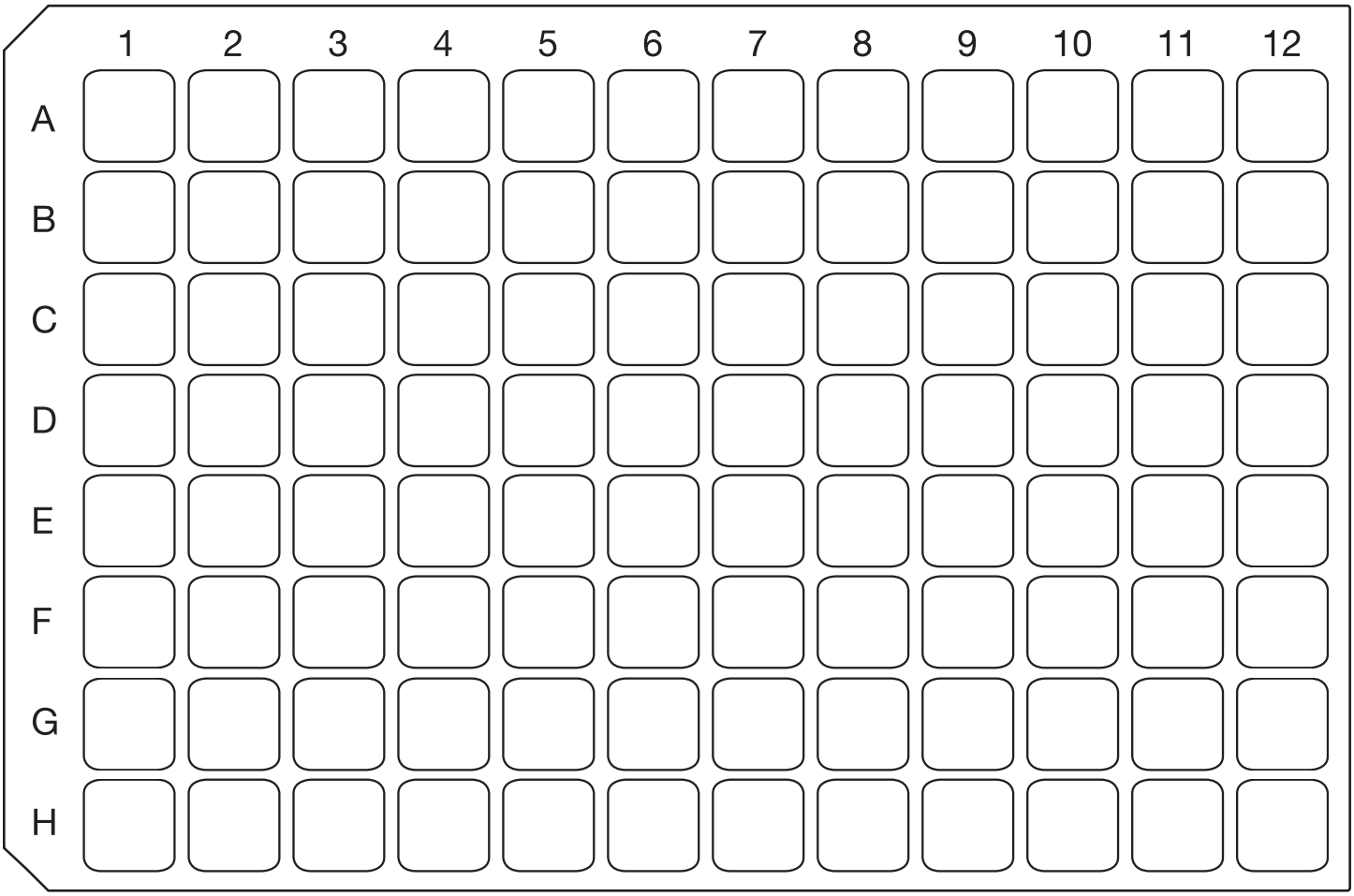
Materials

- 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., #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
- 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 Indirect ELISA

1. Coat microtiter plate wells with 100 µl of the antigen solution 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 300 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 unconjugated detection antibody (appropriately diluted in wash buffer) to each well. Incubate for 1 hr at 37°C. Wash three times in wash buffer.
5. Add 100 µl of enzyme-conjugated secondary 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 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.
7. 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.

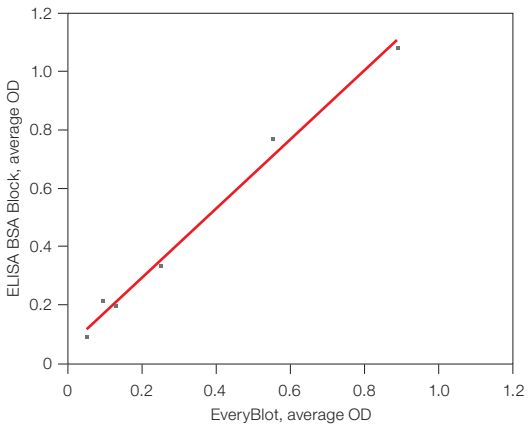
Microplate Layout



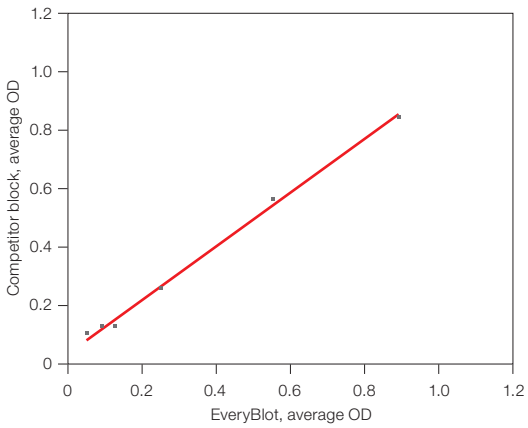
Comparison of Blocking Buffers

Antigen Concentration, pg	Absorbance Values, OD					
	ELISA BSA Block		EveryBlot Blocking Buffer		Competitor Block	
400	2.048	2.056	1.447	1.344	1.406	1.262
200	1.117	0.962	0.608	0.586	0.605	0.552
100	0.574	0.576	0.35	0.304	0.343	0.339
50	0.316	0.317	0.204	0.199	0.197	0.183
25	0.246	0.214	0.162	0.136	0.163	0.15
0	0.064	0.065	0.08	0.057	0.084	0.073
	▪ 150 µl/well ▪ Incubation time: 1 hr		▪ 300 µl/well ▪ Incubation time: 0 min		▪ 300 µl/well ▪ Incubation time: 0 min	

EveryBlot Blocking Buffer vs. ELISA BSA Block



EveryBlot Blocking Buffer vs. Competitor Block



Blocking buffer comparison. EveryBlot Blocking Buffer versus the standard ELISA BSA Block (Bio-Rad, #BUF032) or competitor ELISA blocking buffer showed comparable results with good correlation coefficients (r = 0.99 with ELISA BSA Block and r = 1.00 with competitor block). Fit line (—). BSA, bovine serum albumin.

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

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