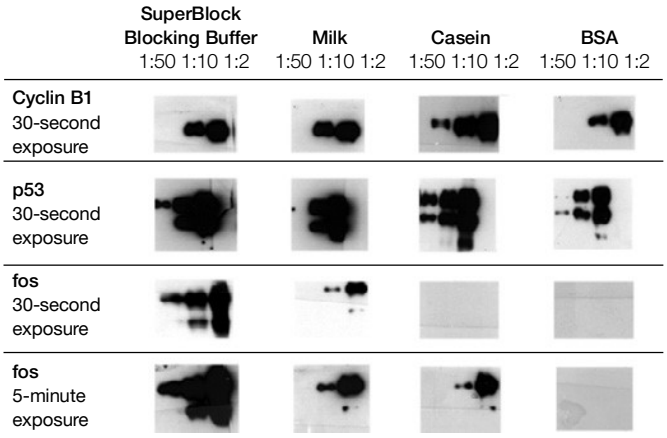

Figure 9. Blocking buffer optimization. Recombinant human cyclin B1, wild-type p53 and mouse fos baculovirus lysates were diluted in Lane Marker Reducing Sample Buffer (1:50, 1:10 or 1:2) and separated electrophoretically on a 12% SDS-polyacrylamide gel. The proteins were transferred to nitrocellulose membrane and cut into strips. The membrane strips were blocked for one hour at room temperature with shaking in Blocker Casein in TBS, 1% BSA in TBS, SuperBlock Blocking Buffer in TBS or 5% nonfat milk in TBS. Tween-20 (0.05%) was added to all blocking buffers. The membranes were then incubated with the appropriate primary antibody at 0.5µg/mL prepared in the different blocking solutions for one hour at room temperature with shaking. Each membrane strip was washed with TBS followed by a one-hour incubation in HRP-conjugated goat anti-mouse antibody prepared in the different blocking buffers at 25ng/mL. The membranes were washed with TBS. A working solution of SuperSignal West Pico Chemiluminescent Substrate was prepared and added to each membrane for five minutes. The membranes were placed in sheet protectors and exposed to film for 30 seconds and five minutes as indicated. The film was developed per the manufacturer's instructions.

Did you know?

BLOTTO is an acronym for Bovine Lacto Transfer Technique Optimizer.

Table 3. Western blot blocking buffers selection guide.

	Product	Description
Start with	StartingBlock Blocking Buffer	Single purified protein; fast blocking; broad applicability; excellent for reblocking stripped blots available in PBS and TBS with and without T20. Compatible with most antibodies as well as biotin-binding reagents
	SEA BLOCK Blocking Buffer	Steelhead salmon serum. Recommended for fluorescence. For reduced cross-reactivity and high signal-to-noise ratios
	SuperBlock Blocking Buffer	Free of biotin and albumin allowing for reduced undesirable binding and high signal-to-noise ratios. Single purified glycoprotein; fast blocking; broad applicability; stabilizes plate-coated antibodies for drying; available in PBS and TBS with and without Tween-20
Next	BSA Blocking Buffer	Purified bovine serum albumin in PBS or TBS
	Casein Blocking Buffer	Purified casein in PBS or TBS
Specialty	Pierce Fast Blocking Buffer	Developed especially for rapid, 5-minute blocking of Western blots as part of the Fast Western Blot system; proprietary proteins formulation in TBS
	Pierce Protein-Free Blocking Buffers	Proprietary non-protein blocking compound; available in PBS and TBS with and without Tween-20. Eliminate potential cross-reactivity associated with protein-based blockers and provide high signal-to-noise ratios
Generic	BLOTTO Blocking Buffer	Non-fat dry milk proteins in TBS
	Clear Milk Blocking Buffer	Milk proteins, clarified and stabilized in proprietary solution

StartingBlock Blocking Buffers

Although no blocking buffer is ideal for every system, you can improve the odds dramatically with Thermo Scientific™ StartingBlock™ Blocking Buffer because it is compatible with the widest variety of antibodies. For example, StartingBlock Blocking Buffers are compatible with biotin-containing systems, while milk-based protein blockers interfere. StartingBlock buffers rarely cross-react with rabbit antibodies, while many other blockers do. StartingBlock Blocking Buffers are also free of potentially interfering serum proteins.

StartingBlock Blocking Buffers offer a high level of performance — regardless of the system you choose for your Western blotting (Figure 10). They may be the only blockers you ever use.

Features:

- **Compatible with a wide range of detection systems** — serum-free, biotin-free and rarely cross-reacts with rabbit antibodies
- **Shorter blocking times** — 1–15 minutes for Western blots
- **Strip and reprobe** — no reblocking necessary

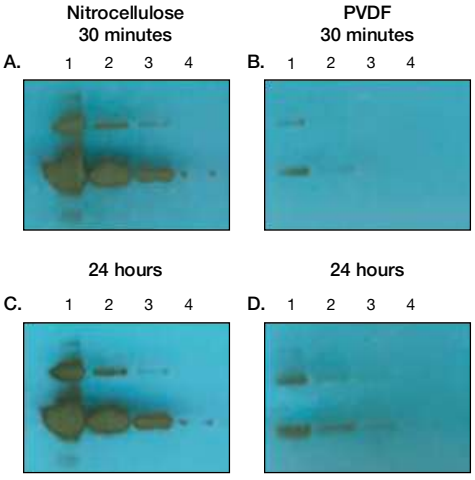

Figure 10. StartingBlock Blocking Buffer performance after stripping and reprobing. Nitrocellulose and PVDF membranes that had been blocked with StartingBlock Blocking Buffer and used to detect a different primary target were then stripped with Restore Western Blot Stripping Buffer (Cat. No. 21059) and probed for the transferrin receptor (CD71). Detection was with SuperSignal West Dura Extended Duration Substrate (Cat. No. 34075) and exposed to film for 30 minutes and then again for 24 hours. Very little background occurs with either membrane or exposure time, indicating exceptional blocking performance.

For ordering information refer to page 78.

SEA BLOCK Blocking Buffer

Thermo Scientific™ SEA BLOCK™ Blocking Buffer is steelhead salmon serum in PBS that is especially useful as a blocking agent in immunohistochemistry (IHC) and other detection methods involving mammalian samples.

Fish serum has several advantages over typical blocking buffers. Because salmon is phylogenetically distant from mammals, which are the source of antibodies and samples used in most experiments, its serum proteins are less likely to have specific binding interactions with proteins used in typical cell biology experiments. When normal serum causes background in immunohistochemistry (IHC), try this product. SEA BLOCK Blocking Buffer is particularly effective in applications involving fluorescence imaging. The blocker has been used to decrease background and increase signal-to-noise ratios with near-IR fluorescent probes and is validated for use with the LI-COR Odyssey Infrared Imaging System.

Features:

- **Non-mammalian** — fish proteins are less likely to have specific binding interactions with antibodies and other mammalian proteins present in typical methods
- **Convenient** — filtered and stabilized in PBS for compatibility with most assay systems
- **Easy to use** — can be used as supplied or diluted up to 10-fold as needed
- **Flexible** — may be used for many different applications, including as a diluent for antibodies
- **Fluorescence optimized** — recommended blocker for fluorescent Western detection

SuperBlock Blocking Buffers

Our most popular biotin-free blocking buffer, Thermo Scientific™ SuperBlock™ Blocking Buffer, now comes in both dry and liquid formats. Many researchers have discovered that SuperBlock Blocking Buffer is the only blocking buffer needed for all of their applications. Each packet of Thermo Scientific™ SuperBlock™ Dry Blend (TBS) Blocking Buffer produces 200mL of buffer.

Features:

- **Fast** — blocks Western blot membranes in five to 10 minutes
- **High sensitivity** — non-serum protein solution yields a high signal-to-noise ratio
- **Flexible formats** — liquid formulations available in PBS or TBS; powder formulation in TBS
- **Compatibility** — biotin-free

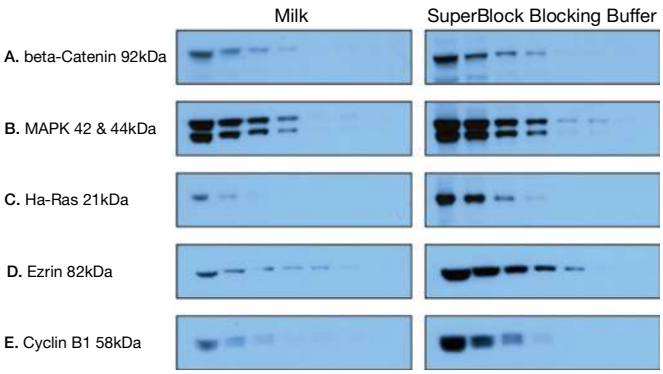

Figure 11. SuperBlock Blocking Buffer in PBS is better than milk for sensitive detection of target proteins. HeLa cell lysate (20, 10, 5, 2.5, 1.25, 0.625 and 0.3125µg) was separated by SDS-PAGE, and transferred to nitrocellulose (Panels A-C, Cat. No. 88014) or PVDF (Panels D-E, Cat. No. 88585) membrane. Membranes were blocked for one hour using 5% milk in Tris-buffered saline with 0.05% Tween-20 Detergent or SuperBlock Blocking Buffer in phosphate-buffered saline with 0.05% Tween-20 Detergent. Membranes were probed for the indicated targets. Blots were incubated in SuperSignal West Pico Chemiluminescent Substrate (Cat. No. 34080) for five minutes and exposed to Thermo Scientific™ CL-XPosure™ Film (Cat. No. 34091).

Blocker BSA

Thermo Scientific™ Blocker BSA in PBS or TBS is a ready-to-use, 10X solution of bovine serum albumin protein for blocking steps in Western blot detection methods. Blocker BSA is usually more effective than nonfat milk blocking buffers for biotin-avidin systems because it contains a single purified protein that is devoid of endogenous biotin.

Features:

- **Purified protein** — 10% solutions of high-quality bovine serum albumin
- **Convenient** — concentrated formulation saves storage space
- **Easy to use** — no powder to dissolve; ready-to-dilute liquid concentrate
- **Flexible** — available in PBS or TBS formulations

Blocker Casein

Thermo Scientific™ Blocker Casein is a ready-to-use, in either TBS or PBS. The blocking buffer contains casein protein that is purified from milk by the Hammarsten method. Blocker Casein Buffers are 1% (w/v) casein, which corresponds to the optimal concentration for most applications. Casein is not recommended for use when probing for phosphoproteins.

Features:

- **Purified casein** — single-protein blocking buffer provides fewer chances of cross-reaction with assay components than serum or milk solutions
- **Easy to use** — 1% casein solutions are ready to use; can be diluted further as needed
- **Flexible** — available in PBS and TBS formulations to suit a variety of applications
- **Safe** — stable, thimerosal-free formulations

Pierce Fast Blocking Buffer

Thermo Scientific™ Pierce™ Fast Blocking Buffer effectively and reliably blocks Western blots in just five minutes to provide low-background results and speed up traditional blotting protocols. The buffer is compatible with antibodies and biotin-avidin systems, as well as both nitrocellulose and PVDF membranes.

Features:

- **Fast** — shorten the typical Western blot development by up to one hour
- **Simple** — optimized protocol makes Western blot analysis easier than ever
- **Low background** — provides results comparable to classic Western blotting buffers

Protein-Free Blocking Buffers

Traditional blocking buffers contain proteins that can cross-react with a system, resulting in high background and reduced signal (Figure 12). Thermo Scientific™ Pierce™ Protein-Free Blocking Buffers eliminate or minimize cross-reactivity associated with protein-based blocking buffers in ELISA, Western blotting, arrays and other immunodetection applications. This buffer is especially useful when other protein-based blocking buffers have resulted in higher background noise.

Features:

- **Protein-free** — eliminate or minimize cross-reactivity associated with protein-based blocking buffers
- **Compatible with multiple detection systems** — can be used in Western blots, ELISA or arrays; does not interfere with avidin-biotin systems
- **High signal-to-noise** — for optimal sensitivity
- **Ready to use** — 1X formulation
- **Saves time and money** — available with 0.05% Tween-20 detergent already added

For ordering information refer to page 78.

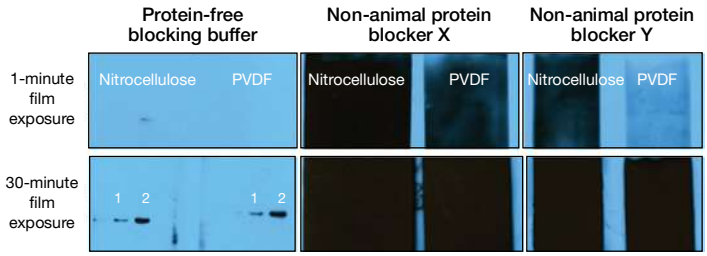

Figure 12. Pierce Protein-Free Blocking Buffer efficiently blocks Western blotting membranes. Jurkat apoptotic lysate (Lane 1: 0.25µg; lane 2: 0.50µg) was separated in 4–20% Tris-glycine gels and transferred to nitrocellulose or PVDF membranes. The membranes were blocked for one hour at room temperature with the indicated blocking buffer, probed with mouse anti-PARP (0.25µg/mL) followed by goat anti-mouse HRP (4ng/mL) and detected by SuperSignal West Dura substrate.

Blocker BLOTTO

Thermo Scientific™ Blocker BLOTTO Blocking Buffer is a ready-to-use 5% solution of nonfat powdered milk in Tris-buffered saline for Western blot and other detection methods.

Features:

- **Convenient** — supplied as a ready-to-use 1X TBS solution; can be diluted as needed
- **Easy to use** — formulated with antifoaming agent and thimerosal-free preservative
- **Flexible** — may be used for multiple applications, including as a diluent for antibody
- **Popular** — nonfat milk has been used for many years in a variety of protein methods, although it is not recommended for avidin-based techniques because it contains some endogenous biotin

Pierce Clear Milk Blocking Buffer

Thermo Scientific™ Pierce™ Clear Milk Blocking Buffer (10X) is a proprietary preformulated milk solution for blocking excess nonspecific binding sites, reducing background in Western blotting applications and diluting antibodies when used with nitrocellulose and PVDF membranes. It is similar to Blocker BLOTTO Buffer, but offers a space-saving feature as 10X concentrate. Pierce Clear Milk Blocking Buffer (10X) provides lower background, enhanced sensitivity, extended shelf life and reproducible results compared to homemade buffers based on dissolved nonfat dry milk.

Features:

- **Excellent stability** — stable for one year stored at 4°C, unlike typical homemade milk buffers
- **Convenient** — concentrated formulation saves storage space and can be diluted easily to obtain optimal blocking results for specific applications
- **Easy to use** — no waiting for powdered milk to dissolve with this ready-to-dilute solution
- **Popular** — nonfat milk has been used for many years in a variety of protein methods, although it is not recommended for avidin-based techniques because it contains some endogenous biotin

Did you know?

Milk, the most popular blocking buffer additive, should not be used with avidin-biotin detection because the biotin in milk will result in high background.

Ordering information

Product	Quantity	Cat. No.
Block, wash, probe		
Automated		
iBind Flex Western Starter Kit	1 kit	SLF2000S
iBind Flex Western Device	1 device	SLF2000
iBind Flex Cards	10 cards	SLF2010
iBind Flex FD Solution Kit	1 kit	SLF2019
iBind Flex Solution Kit	1 kit	SLF2020
iBind Western Starter Kit	1 kit	SLF1000S
iBind Western Device	1 device	SLF1000
iBind Cards	10 cards	SLF1010
iBind FD Solution Kit	1 kit	SLF1019
iBind Solution Kit	1 kit	SLF1020
Blocking solutions		
Membrane Blocking Solution	1L	00-0105
WesternBreeze Blocker/Diluent (Part A and B)	80mL	WB7050
Blocker BLOTTO in TBS	1L	37530
Blocker BSA in PBS (10X)	200mL	37525
Blocker BSA in TBS (10X)	125mL	37520
Blocker Casein in PBS	1L	37528
Blocker Casein in PBS	100mL	37582
Blocker Casein in TBS	1L	37532
Pierce Clear Milk Blocking Buffer (10X)	100mL	37587
Pierce Fast Blocking Buffer	500mL	37575
Pierce Fast Blocking Buffer	100mL	37576
Blocker Casein in TBS	100mL	37583
Pierce Protein-Free (PBS) Blocking Buffer	1L	37572
Pierce Protein-Free (PBS) Blocking Buffer	100mL	37584

Product	Quantity	Cat. No.
Pierce Protein-Free (TBS) Blocking Buffer	1L	37570
Pierce Protein-Free (TBS) Blocking Buffer	100mL	37585
Pierce Protein-Free T20 (PBS) Blocking Buffer	1L	37573
Pierce Protein-Free T20 (TBS) Blocking Buffer	1L	37571
SEA BLOCK Blocking Buffer	500mL	37527
SEA BLOCK Blocking Buffer	3 x 500mL	37527X3
StartingBlock (PBS) Blocking Buffer	1L	37538
StartingBlock (PBS) Blocking Buffer	100mL	37578
StartingBlock (TBS) Blocking Buffer	1L	37542
StartingBlock (TBS) Blocking Buffer	100mL	37579
StartingBlock T20 (PBS) Blocking Buffer	1L	37539
StartingBlock T20 (TBS) Blocking Buffer	1L	37543
SuperBlock (PBS) Blocking Buffer	1L	37515
SuperBlock (PBS) Blocking Buffer	5L	37518
SuperBlock (PBS) Blocking Buffer	100mL	37580
SuperBlock (PBS) Blocking Buffer - Blotting	1L	37517
SuperBlock (TBS) Blocking Buffer	1L	37535
SuperBlock (TBS) Blocking Buffer	100mL	37581
SuperBlock (TBS) Blocking Buffer - Blotting	1L	37537
SuperBlock (TBS) Blocking Buffer Dry Blend	5 packs	37545
SuperBlock T20 (PBS) Blocking Buffer	1L	37516
SuperBlock T20 (TBS) Blocking Buffer	1L	37536
I-Block Protein-Based Blocking Reagent	30g	T2015