

Washing the membrane

Like other immunoassay procedures, Western blotting consists of a series of incubations with different antibodies separated by wash steps. Washing steps are necessary to remove unbound or weakly bound reagents and to reduce background, increasing the signal-to-noise ratio. Insufficient washing produces high background, while excessive washing may result in decreased sensitivity caused by elution of the antibody and/or antigen from the blot. As with other steps in performing a Western blot, a variety of buffers may be used. Occasionally, washing is performed in a physiological buffer such as Tris-buffered saline (TBS) or phosphate-buffered saline (PBS) without any additives. More commonly, a detergent such as 0.05% Tween-20 Detergent (Cat. No. 28320) is added to the buffer to help remove nonspecifically bound material. Another common technique is to use a dilute solution of the blocking buffer along with some added detergent to help minimize background.

Although detergents are available from several commercial sources and used routinely in many research laboratories, the importance of detergent purity and stability is not widely appreciated. Detergents often contain trace impurities from their manufacture. Some of these impurities, especially peroxides that are found in most nonionic detergents, will destroy protein activity. In addition, several types of detergents oxidize readily when exposed to the air or UV light, causing them to lose their properties and potency as solubilizing agents. We offer several high-purity, low peroxide-containing detergents that are packaged under nitrogen gas in clear glass ampules. These Thermo Scientific™ Surfact-Amps™ Detergent Solutions provide unsurpassed convenience, quality and consistency for all detergent applications. Commonly used detergents for Western blotting include Tween-20 and NP-40 detergent solutions.

BupH Phosphate Buffered Saline Packs (PBS)

Each pack of Thermo Scientific™ BupH™ Phosphate Buffered Saline yields 500mL of 0.1M phosphate, 0.15M sodium chloride, pH 7.0 when dissolved in 500mL deionized water (20L total).

BupH Tris Buffered Saline (TBS)

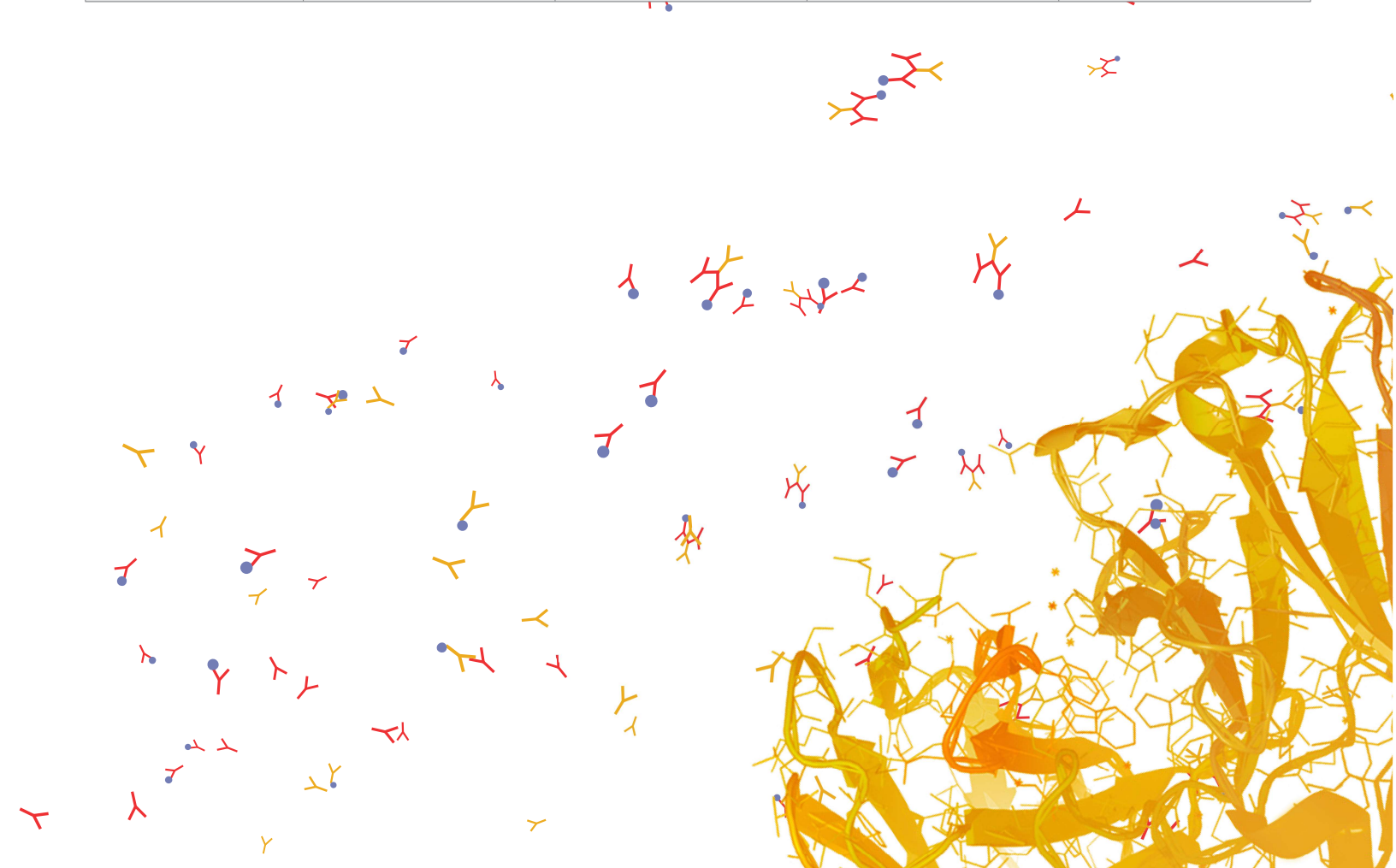
Each pack of Thermo Scientific™ BupH™ Tris Buffered Saline yields 500mL of 25mM Tris, 0.15M sodium chloride, pH 7.2 when dissolved in 500mL deionized water (10 packs make 5L total; 40 packs make 20L total).

Tween-20 Surfact-Amps Detergent Solution

Thermo Scientific™ Tween™-20 Surfact-Amps™ Detergent Solution is a highly-purified Tween-20 detergent stabilized as a 10% solution and packaged under nitrogen in glass ampules or non-leaching high-density polyethylene (HDPE) bottles, ensuring its stability and eliminating the accumulation of peroxides and degradation products.

Table 5. Wash buffer selection guide.

	TBS	PBS	TBST	PBST
Dry blend	28376 — 40 packs 28379 — 10 packs	28372 — 40 packs		
Liquid concentrate	28358 — 500mL	28348 — 500mL	28360 — 500mL	28352 — 500mL
Formulation	Both dry and liquid: <ul style="list-style-type: none">• 25mM Tris• 0.15M NaCl• pH 7.2	Dry: <ul style="list-style-type: none">• 0.1M sodium phosphate• 0.15M NaCl• pH 7.2 Liquid: <ul style="list-style-type: none">• 10mM sodium phosphate• 0.15M NaCl• pH 7.5	<ul style="list-style-type: none">• 25mM Tris• 0.15M NaCl• 0.05% Tween-20• pH 7.5	<ul style="list-style-type: none">• 10mM sodium phosphate• 0.15M NaCl• 0.05% Tween-20• pH 7.5
Application	<ul style="list-style-type: none">• ELISA, Western blotting and other immunoassays• Diluent or blocking buffer for plate-based assays	<ul style="list-style-type: none">• ELISA, Western blotting and other immunoassays• Crosslinking, biotinylation and fluorescent labeling reactions requiring an amine-free buffer	<ul style="list-style-type: none">• ELISA, Western blotting and other immunoassays• Diluent or blocking buffer for plate-based assays	<ul style="list-style-type: none">• ELISA, Western blotting and other immunoassays• Diluent or blocking buffer for plate-based assays



Ordering information

Product	Quantity	Cat. No.
Secondary Abs and solutions		
Stabilized Goat Anti-Mouse IgG (H+L), Peroxidase Conjugated (10µg/mL)	2mL	32430
Stabilized Goat Anti-Rabbit IgG (H+L), Peroxidase Conjugated (10µg/mL)	2mL	32460
Pierce High Sensitivity Streptavidin-HRP (1mg/mL)	0.5mL	21130
Pierce High Sensitivity Streptavidin-HRP (1mg/mL)	5mL	21132
Pierce High Sensitivity Streptavidin-HRP, pre-diluted (10µg/mL)	2mL	21134
Pierce High Sensitivity NeutrAvidin-HRP (1mg/mL)	0.5mL	31030
Pierce High Sensitivity NeutrAvidin-HRP	5mL	31032
Goat Anti-Mouse IgG (H+L), Peroxidase Conjugated	2.0mL	31430
Goat Anti-Rabbit IgG (H+L), Peroxidase Conjugated	2.0mL	31460
Pierce Goat Anti-Mouse Poly-HRP (0.5mg/mL)	0.5mL	32230
Pierce Goat Anti-Rabbit Poly-HRP (0.5mg/mL)	0.5mL	32260
Ethylene Glycol (50% solution)	200mL	29810
Pierce Peroxidase Conjugate Stabilizer	25mL	31503
Guardian Peroxidase Conjugate Stabilizer/Diluent	200mL	37548
Guardian Peroxidase Conjugate Stabilizer/Diluent	1L	37552
Protein Stabilizing Cocktail	10mL	89806
HRP-Conjugate Diluent/Stabilizer	1L	00-2018
Pierce Stable Peroxide Substrate Buffer (10X)	100mL	34062
Pierce Peroxidase Conjugate Stabilizer	25mL	31503
Poly-HRP Dilution Buffer	1L	N501
Poly-HRP Dilution Buffer	100mL	N500

Product	Quantity	Cat. No.
Wash buffers		
Pierce Fast Wash Buffer, 10X	1L	37577
Wash Buffer (25X)	100mL	WB01
Wash Buffer (25X)	1L	WB02
BupH Phosphate Buffered Saline Packs	40 packs	28372
BupH Tris Buffered Saline Packs	40 packs	28376
BupH Tris Buffered Saline Packs	10 packs	28379
PBS (Phosphate-Buffered Saline) Powder	12 x 1L	00-3000
PBS (Phosphate-Buffered Saline) Tablets	100 tablets	00-3002
PBS (Phosphate-Buffered Saline) (10X) pH 7.4	500mL	AM9624
PBS (Phosphate-Buffered Saline) (10X) pH 7.4	1L	AM9625
Pierce 20X PBS Tween-20 Buffer	500mL	28352
Pierce 20X Phosphate Buffered Saline	500mL	28348
Pierce 20X TBS Buffer	500mL	28358
Pierce 20X TBS Tween-20 Buffer	500mL	28360
TBS Buffer	1 pack	R017R.0000
WesternBreeze Wash Solution (16X)	2 x 100mL	WB7003