Simple Western Size Assay Buffer Compatibility (Jess, Abby, Wes, Sally Sue, and Peggy Sue)

Lysis Buffer Compatibility Table

LYSIS BUFFER	USAGE	VENDOR AND CATALOG	RANGE TESTED*	CHEM SIGNAL	CHEM RESOLUTION	FLUORESCENT STANDARDS MW SIZING	RECOMMENDATIONS
T-PER	Tissue Protein	Thermo 78510	50–90%	• 12–230 kDA: Slight signal decrease ≥50% • 66–440 kDA: Slight signal decrease ≥50%	• 2–40 kDA: No effect at 75% • 66–440 kDA: Resolution decrease at >50%	• 2–40 kDA: No effect at 75% • 66–440 kDA: Signal decrease and smearing at >50%	• 12–230 kDA: For best signal, dilute 1:2 in 0.1X Sample Buffer • 66–440 kDA: For best results, dilute 1:3 in 0.1X Sample Buffer
M-PER	Mammalian cells	Thermo 78503	• 2–40 kDA: 75% • 12–230 kDA: 50–900% • 66–440 kDA: 50–900%	No effect	No effect	No effect	Samples can be used undiluted with Master Mix
RIPA	General Whole Cell Lysis Buffer	Cell Signalling 9806	0–90%	• 2–40 kDA: Increase signal at 75% • 12–230 kDA: Signal decrease at 90% • 66–440 kDA: Signal loss at >50%	• 2–40 kDA: Peak broadening <10 kDA at 75% • 66–440 kDA: Resolution decreases at >50%	• 2–40 kDA: Compression of standards at 75% • 12–230 kDA: 230 kDA signal decrease. Non-linear compression causing 1 kDA and 29 kDA standards to run closer • 66–440 kDA: Signal decrease and smearing at 50%	• 2–40 kDA: Prepare Biotinylated Ladder in the same buffer for consistent sizing. Separation time may be increased to offset compression of standards • 12–230kDa: For best signal and resolution, dilute 1:2 in 0.1X Sample Buffer • 66–440 kDA: Dilute at least 1:3
Cell Lysis Buffer	General Whole Cell Lysis Buffer	Cell Signalling 9803	0–90%	• 2–40 kDA: Increase in signal at 75% • 12–230 kDA: Slight signal decrease at high MW region • 66–440 kDA: Signal loss at >50%	• 2–40 kDA: Peak broadening <10 kDA at 75% • 66–440 kDA: Resolution decrease at >50%	• 2–40 kDA: Compression of standards at 75% • 12–230 kDA: 230 kDA signal decrease. Non-linear compression causing 1 kDA and 29 kDA standards to run closer • 66–440 kDA: Signal decrease in 280 kDA at >50%	• 2–40 kDA: Prepare Biotinylated Ladder in the same buffer for consistent sizing. Separation time may be increased to offset compression of standards • 12–230kDa: For best signal and resolution, dilute 1:2 in 0.1X Sample Buffer • 66–440 kDA: Dilute at least 1:3
SDS Lysis Buffer	General Whole Cell Lysis Buffer	Millipore #20–163	0–90%	No effect	No effect	• 2–40 kDA: Significant signal decrease for 1 kDA standard and registration due to peak splitting at 75% • 12–230 kDA: Smearing of Std 1 peak	• 12–230 kDA: For best signal and resolution, dilute 1:2 in 0.1X Sampl Buffer. Prepare Biotinylated Ladder in same sample buffer for consistent sizing
IP Lysis Buffer	General Whole Cell Lysis Buffer	Thermo 87787	0–90%	• 2–40 kDA: Slight signal increase at 75% • 12–230 kDA: Slight signal decrease at high MW region • 66–440 kDA: Signal decrease at >50%	• 2–40 kDA: Peak broadening <10 kDA at 75% • 66–440 kDA: Resolution decreases at >50%	• 2–40 kDA: Compression of standards at 75% • 12–230 kDA: Improved fluorescent standard signal and resolution • 66–440 kDA: Signal decrease and smearing in 280 kDA standard at >20%	• 2–40 kDA: Prepare Biotinylated Ladder in the same buffer for consistent sizing. Separation time may be increased to offset compression of standards • 12–230 kDA: From best signal and resolution, dilute 1:2 in 0.1X Sample Buffer. Prepare Biotinylated Ladder ins same sample buffer for consistent sizing • 66–440 kDA: Dilute 1:3

*All results compared against ProteinSimiple Bicine/CHAPS Buffer.
All results apply to Simple Western size assays with Split Running Buffer (RB) 2 (12–230 kDA), Split RB3 (66–440 kDA), Split RB4 (2–40 kDA) as indicated for each lysis buffer or buffer component tested.
Unless otherwise stated for each separation matrix, the chemi signal, resolution, fluorescent standards, and molecular weight sizing were not affected by the use of addition of the listed components in the ranges tested.

Simple Western Size Assay Buffer Compatibility (Jess, Abby, Wes, Sally Sue, and Peggy Sue)

Lysis Buffer Compatibility Table, continued

LYSIS BUFFER	USAGE	VENDOR AND CATALOG	RANGE TESTED*	CHEM SIGNAL	CHEM RESOLUTION	FLUORESCENT STANDARDS MW SIZING	RECOMMENDATIONS
Cellytic MT	General Whole Cell Lysis Buffer	Sigma C3228	0–90%	• 2–40 kDA: No effect at 75% • 66–440 kDA: Signal decrease at 90%	• 2–40 kDA: No effect at 75% • 66-400 kDA: Resolution decrease at 90%	• 2–40 kDA: No effect at 75% • 12–230 kDA: Improved Fluorescent Standard signal and resolution • 66–440 kDA: Signal and resolution decrease in 280 kDA at 90%	• 12–230 kDA: Samples can be used undiluted with Master Mix • 66–440 kDA: For best results, dilute 1:2 in 0.1X Sample Buffer

Lysis Buffer Compatibility Table (for Fluorescence Detection Only)

LYSIS BUFFER	USAGE	VENDOR AND CATALOG	RANGE TESTED*	FLUORESCENT SIGNAL	FLUORESCENT RESOLUTION	FLUORESCENT STANDARDS MW SIZING	RECOMMENDATIONS	PROTEIN NORMALIZATION
T-PER	Tissue Protein	Thermo 78510	100%	• 12–230 kDA: Slight signal decrease • 66–440 kDA: Slight signal decrease	• 2–40 kDA: No effect • 66–440 kDA: Slight resolution decrease	• 2–40 kDA: No effect • 66–440 kDA: Signal decrease and smearing		• 2–40 kDa: No effect • 12–230 kDa: No effect • 66–440 kDa: No effect
RIPA	General Whole Cell Lysis Buffer	Cell Signalling 9806	100%	• 2–40 kDA: Increase in signal at highest concentration of buffer • 12–230 kDA: Signal decrease • 66–440 kDA: Signal loss	• 2–40 kDA: Peak broadening such that targets ≤10 kDa may be difficult to detect • 66–440 kDA: Resolution decrease	• 2–40 kDA: Compression of standards—1 kDa and 26 kDa run closer • 12–230 kDA: 1 kDa and 29 kDa stds run closer • 66–440 kDA: Signal decrease and smearing	Prepare biotinylated ladder in the same buffer for consistent sizing. Separation time may be increased to offset compression of standards.	• 2–40 kDA: Compression of standards causes PN curve to shift but no effect on signal. • 12–230 kDA: No effect • 66–440 kDA: No effect
Cell Lysis Buffer	General Whole Cell Lysis Buffer	Cell Signalling 9803	100%	• 2–40 kDA: Increase in signal at highest concentration of buffe • 12–230 kDA: Signal decrease • 66–440 kDA: Signal loss	• 2–40 kDA: Peak broadening such that targets ≤10 kDA may be difficult to detect • 66–440 kDA: Resolution decrease	• 2–40 kDA: Compression of standards – 1 kDA and 26 kDA run closer • 12–230 kDA: 1 kDA and 29 kDA stds run closer • 66–440 kDA: Signal decrease and smearing	Prepare biotinylated ladder in the same buffer for consistent sizing. Separation time may be increased to offset compression of standards.	• 2–40 kDA: compression of standards causes PN curve to shift but no effect on signal. • 12–230 kDA: No effect • 66–440 kDA: No effect
Pierce IP Lysis Buffer	General Whole Cell Lysis Buffer	Thermo 87787	100%	• 2–40 kDA: Increase in signal at highest concentration of buffer • 12–230 kDA: Signal decrease • 66–440 kDA: Signal loss	• 2–40 kDA: Peak broadening such that targets ≤10 kDA may be difficult to detect • 66–440 kDA: Resolution decrease	• 2–40 kDA: Compression of standards—1 kDA and 26 kDA run closer • 66–440 kDA: Signal decrease and smearing in Std 280 kDA	Prepare biotinylated ladder in the same buffer for consistent sizing. Separation time may be increased to offset compression of standards.	• 2–40 kDA: compression of standards causes PN curve to shift but no effect on signal. • 12–230 kDA: No effect • 66–440 kDA: No effect

*100% of available volume which translates to 76% of absolute volume

Simple Western Size Assay Buffer Compatibility (Jess, Abby, Wes, Sally Sue, and Peggy Sue)

Buffer Reagents

COMPONENT	TEST RANGE	COMPATIBLE RANGE	CHEMI SIGNAL	CHEMI RESOLUTION	FLUORESCENT STANDARDS	MW SIZING
Bicine	• 2–40 kDa: 12.5–50 mM • 12–230 kDa: 20–50 mM	• 2–40 kDa: 12.5–50 mM • 12–230 kDa: 20–50 mM	No effect	No effect	No effect	No effect
TrisCl, pH 7.5	• 2–40 kDa: 12.5–50 mM • 12–230 kDa: 10–50 mM	• 2–40 kDa: 12.5–50 mM • 12–230 kDa: 10–50 mM	No effect	No effect	No effect	No effect
HEPES, pH 8.0	• 2–40 kDa: 12.5–50 mM • 12–230 kDa: 10–50 mM	• 2–40 kDa: 12.5–50 mM • 12–230 kDa: 10–50 mM	2–40 kDa: Signal increase ≥25 mM	2–40 kDa: Decrease in resolution ≥25 mM	No effect	No effect
Sodim Phosphate (NaH_2PO_4/ Na_2HPO_4)	• 2–40 kDa: 7.5–30 mM • 12–230 kDa: 10–30 mM	• 2–40 kDa: 12.5–50 mM • 12–230 kDa: 10–50 mM	No effect	No effect	No effect	No effect
MES, pH 6.7	• 2–40 kDa: 1.25 - 100 mM	• 2–40 kDa: 1.25 - 100 mM	No effect	No effect	No effect	No effect

Buffer Reagents (for Fluorescence Detection Only)

COMPONENT	TEST RANGE	COMPATIBLE RANGE	FLUORESCENT SIGNAL	FLUORESCENT RESOLUTION	FLUORESCENT STANDARDS	MW SIZING	PROTEIN NORMALIZATION
HEPES, pH 8.0	12.5–50 mM	12.5–50 mM	• 2–40 kDa: Increase in signal ≥25 mM • 12–230 kDa: Signal decrease ≥25 mM	• 2–40 kDa: Decrease in resolution ≥25 mM	No effect	No effect	No effect in 2–40 kDa, 12–23 kDa and 66–440 kDa
MES, pH 6.7	1.25–100 mM	1.25–100 mM	No effect	No effect	No effect	No effect	No effect in 2–40 kDa, 12–23 kDa and 66–440 kDa

Dyes

COMPONENT	TEST RANGE	COMPATIBLE RANGE	CHEMI SIGNAL	CHEMI RESOLUTION	FLUORESCENT STANDARDS	MW SIZING
Bromophenol Blue	0.001–0.01%	0.001–0.01%	• 12–230 kDa: Slight decrease at 0.01% • 66–440 kDa: Decrease at low lysate concentration at >0.1%	• 66–440 kDa: Decrease in resolution in 0.02 mg/mL HeLa at 0.01%	No effect	No effect
Phenol Red	0.05%–0.01%	0.05–0.01%	No effect	No effect	No effect	No effect

Simple Western Size Assay Buffer Compatibility (Jess, Abby, Wes, Sally Sue, and Peggy Sue)

Dyes (for Fluorescence Detection Only)

COMPONENT	TEST RANGE	COMPATIBLE RANGE	FLUORESCENT SIGNAL	FLUORESCENT RESOLUTION	FLUORESCENT STANDARDS	MW SIZING	PROTEIN NORMALIZATION
Phenol Red	0.01%	0.005–0.01%	No effect	No effect	No effect	No effect	No effect in 2–40 kDa, 12–23 kDa and 66–440 kDa

Salts

COMPONENT	TEST RANGE	COMPATIBLE RANGE	CHEMI SIGNAL	CHEMI RESOLUTION	FLUORESCENT STANDARDS	MW SIZING
NaCl	0–700 mM	• 2–40 kDA: 0–150 mM • 12–230 kDA: 0–300 mM • 66–440 kDA: 0–300 mM	• 2–40 kDA: Peak broadening at >150mM • 12–230 kDA: Signal decrease at 300 mM • 66–440 kDA: Low signal >300 mM	• 2–40 kDA: Decrease in resolution >150 mM • 12–230 kDA: Slight decrease at 300 mM • 66–440 kDA: Slight decrease at >300 mM	• 2–40 kDA: Decrease in signal at >150 mM • 12–230 kDA: Std 230 kDA resolution decreases at >300 mM • 66–440 kDA: Std 230 kDA slight smear at >700 mM	• 2–40 kDA: No effect • 12–230 kDA: MW sizing may be affected at high MW region due to 230 kDA standard resolution decrease at >300 mM • 66–440 kDA: No effect
NH4Cl	0–300 mM	• 2–40 kDA: 0–100 mM • 12–230 kDA: 0–150 mM • 66–440 kDA: 0–150 mM	• 2–40 kDA: Peak broadening at >100 mM • 12–230 kDA: Lower signal on high MW end at >150 mM • 66–440 kDA: Lower signal on high MW end at >150 mM	• 2–40 kDA: Decrease in resolution at >100 mM • 12–230 kDA: Loss of resolution >150 mM • 66–440 kDA: Loss of resolution >150 mM	• 12–230 kDA: Std 230 kDA resolution decrease at >75 mM • 66–440 kDA: Std 280 kDA resolution decrease at >75 mM	• 2–40 kDA: No effect • 12–230 kDA: MW sizing may be affected at high MW region due to 230 kDA standard resolution decrease at >75 mM • 66–440 kDA: No effect
$MgCl_2$	0–10 mM	0–10 mM	No effect	No effect	No effect	No effect

Salts (for Fluorescence Detection Only)

COMPONENT	TEST RANGE	COMPATIBLE RANGE	FLUORESCENT SIGNAL	FLUORESCENT RESOLUTION	FLUORESCENT STANDARDS	MW SIZING	PROTEIN NORMALIZATION
NaCl	300 mM	0–150 mM	• 2–40 kDA: Peak broadening at >150 mM • 12–230 kDA: Signal decrease at 300 mM • 66–440 kDA: Low signal >300 mM	• 2–40 kDA: Decrease in resolution at >150 mM • 12–230 kDA: Slight decrease at 300 mM • 66–440 kDA: Slight decrease at 300 mM	• 2–40 kDA: Decrease in signal at >150 mM • 12–240 kDA: Std 230 kDA resolution decrease • 66–440 kDA: Std 280 kDA smearing	No effect	No effect in 2–40 kDA, 12–23 kDA and 66–440 kDa
NH_4Cl	150 mM	0–100 mM	• 2–40 kDA: Peak broadening at >100 mM • 12–230 kDA: Signal decrease at 150 mM • 66–440 kDA: Low signal >150 mM	• 2–40 kDA: Decrease in resolution at >150 mM • 12–230 kDA: Resolution decrease at 150 mM • 66–440 kDA: Resolution decrease at 300 mM	• 12–240 kDA: Std 230 kDA resolution decrease • 66–440 kDA: Std 280 kDA smearing, resolution decrease	No effect	No effect in 2–40 kDA, 12–23 kDA and 66–440 kDa
$MgCl_2$	10 mM	0–10 mM	No effect	No effect	No effect	No effect	No effect in 2–40 kDA, 12–23 kDA and 66–440 kDa

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Reducing Agents

COMPONENT	TEST RANGE	COMPATIBLE RANGE	CHEMI SIGNAL	CHEMI RESOLUTION	FLUORESCENT STANDARDS	MW SIZING
DTT	40 –80 mM	40–80 mM	No effect	No effect	No effect	No effect
βME	25–400 mM	25–400 mM	No effect	No effect	No effect	No effect
TCEP	0.5–2.0mM	• 2–40 kDa: 0.5–20 mM • 12–230 kDa: 0.5 mM • 66–440 kDa: 0.5 mM	No effect	No effect	No effect	No effect

Reducing Agents (for Fluorescence Detection Only)

COMPONENT	TEST RANGE	COMPATIBLE RANGE	FLUORESCENT SIGNAL	FLUORESCENT RESOLUTION	FLUORESCENT STANDARDS	MW SIZING	PROTEIN NORMALIZATION
DTT	80 mM	40–80 mM	No effect	No effect	No effect	No effect	No effect
BME	400 mM	25–400 mM	No effect	No effect	No effect	No effect	No effect
TCEP	20 mM	0.6–20 mM	No effect	No effect	No effect	No effect	No effect

Denaturing Agents

COMPONENT	TEST RANGE	COMPATIBLE RANGE	CHEMI SIGNAL	CHEMI RESOLUTION	FLUORESCENT STANDARDS	MW SIZING
Urea	0–7.6 M	• 2–40 kDa: 0–7.6 M • 12–230 kDa: 0–4 M • 66–440 kDa: 0–2 M	66–440 kDa: Slight decrease at 4 M	66–440 kDa: Slight decrease at 4 M	12–230 kDa: Std 1 slight front end smear, but picked up consistently	No effect
Urea/Thiourea	• 2–40 kDa: 1 M Urea/ 0.2 M Thiourea–5 M Urea/1 M Thiourea • 12–230 kDa: 2 M • Urea/0.4 M Thiourea–5 M Urea/1 M Thiourea • 66–440 kDa: 1 M • Urea/0.2 M Thiourea–5 M Urea/1 M Thiourea	• 2–40 kDa: 1 M Urea/0.2 M Thiourea–5 M Urea/1 M Thiourea • 12–230 kDa: 2 M Urea/0.4 M Thiourea–5 M Urea/1 M Thiourea • 66–440 kDa: 0-2 M Urea/0.4 M Thiourea	66–440 kDa: Slight decrease at 5 M Urea/1 M Thiourea	66–440 kDa: Slight decrease at 5 M Urea/1 M Thiourea	12–230 kDa: Std 1 slight front end smear, but picked up consistently	No effect in range tested

Denaturing Agents (for Fluorescence Detection Only)

COMPONENT	TEST RANGE	COMPATIBLE RANGE	FLUORESCENT SIGNAL	FLUORESCENT RESOLUTION	FLUORESCENT STANDARDS	MW SIZING	PROTEIN NORMALIZATION
Urea	7.6 M	0–7.6 M	No effect	No effect	No effect	No effect	No effect when FRESHLY prepared

Simple Western Size Assay Buffer Compatibility (Jess, Abby, Wes, Sally Sue, and Peggy Sue)

Detergents

COMPONENT	TEST RANGE	COMPATIBLE RANGE	CHEMI SIGNAL	CHEMI RESOLUTION	FLUORESCENT STANDARDS	MW SIZING
Triton X-100	0–2%	0–0.5% (All MW ranges)	• 2–40 kDA: Severe peak broadening at ≥1% • 12–230 kDA: Signal loss >1% • 66-440kDa: Signal loss >0.5%	• 2–40 kDA: Slight resolution loss ≥1% • 12–230 kDA: Slight resolution loss ≥1%	• 2–40 kDA: Peak splitting of 1kDa standard at ≤0.25% • 12–230 kDA: Std 1 front end smeared, but other standards not affected	• 2 –40 kDA: Compression of standards (1 kDA and 26 kDA run closer) at >0.5% • 12–230 kDA: Affects sizing at high MW >0.5% due to 230 kDA standards resolution loss
NP40	0–2%	• 2–40 kDA: 0–0.5% • 12–230 kDA: 0–2% • 66–440 kDA: 0–2%	2–40 kDA: Severe peak broadening at ≥1%	2–40 kDA: Poor resolution	• 2–40 kDA: Peak broadening for Std 1 kDA • 12–230 kDA: Slight smear of Std 1, other Stds not affected	• 2–40 kDA: Compression of standards (1 kDA and 26 kDA run closer) at >0.5% • 66–440 kDA: Affects sizing >2%
Igepal CA 630	• 2–40 kDA: 0–0.2% • 12–230 kDA: 0–1%	• 2–40 kDA: 0–0.2% • 12–230 kDA: 0–1%	No effect	No effect	2–40 kDA: Peak splitting of 1 kDA standard	2–40 kDA: May affect MW sizing for small MW targets due to Std 1 splitting
C7BZO	• 2–40 kDA: 0–1% • 12–230 kDA: 0–1% • 66–440kDa: 0–1%	• 2–40 kDA: 0–0.025% • 12–230 kDA: 0–0.5% • 66–440 kDA: 0–0.5%	• 2–40 kDA: Severe peak broadening at ≥0.5% • 12–230 kDA: Signal loss >1% • 66–440 kDA: Signal decreased at 1% for high MW targets	2–40 kDA: Poor resolution at ≥0.5%	2–40 kDA: Peak broadening for Std 1kDa 12–230 kDA: Slight compression at 1%	• 2–40 kDA: Compression of standards (1 kDA and 26 kDA run closer) at >0.5% • 12–230 kDA: May affect sizing at high MW if >1% due to compression
CHAPS	0.6–2%	0.6–2%	No effect	No effect	No effect	• 2–40 kDA: Compression of standards (1 kDA and 26 kDA run closer) at 2%. Prepare Biotinylated Ladder in the same buffer for consistent sizing. Separation time may be increased to offset compression of standards • 12–230 kDA: Prepare Biotinylated Ladder in same sample buffer for accurate MW sizing
SDS	1–2%	1–2%	2–40 kDA: Peak broadening at 2%	2–40 kDA: Decrease resolution at 2%	• 2–40 kDA: 1kDa Std peak splitting at higher concentrations • 12–230 kDA:1kDa standard slight smear at 1% • 66–440 kDA: No effect or slight Std 1 peak splitting)	May affect sizing due to 1 kDA Std peak splitting
Sodium Deoxycholate	0–1%	• 2–40 kDA: 0–1% • 12–230 kDA: 0–0.5% • 66–440 kDA: 0–0.5%	12–230 kDA: Signal decrease ≥0.5%	No effect	• 2–40 kDA: Peak splitting of 1kDa standard at ≥0.5%	No effect
LDS	• 2–40 kDA: 0.5–2% • 12–230 kDA: 0–1% • 66–440 kDA: 0–1%	0.5–1%	2–40 kDA: Peak broadening at 2%	2–40 kDA: Decrease resolution at 2%	• 2–40 kDA: Peak splitting of 1kDa standard at ≥1% • 12–230 kDA: 1kDa standard slightly smeared ≥1%, but consitently picked up	12–230 kDA: May affect sizing due to Std 1 smear
Sarkosyl	0–1%	• 2–40 kDA: 0–0.5% • 12–230kDa: 0–1% • 66-440kDa: 0–1%	2–40 kDA: Peak broadening at 1%	2–40 kDA: Decrease resolution at 1%	• 2–40 kDA: Peak broadening for 1kDa Std peak	No effect in range tested

Simple Western Size Assay Buffer Compatibility (Jess, Abby, Wes, Sally Sue, and Peggy Sue)

Detergents, continued

COMPONENT	TEST RANGE	COMPATIBLE RANGE	CHEMI SIGNAL	CHEMI RESOLUTION	FLUORESCENT STANDARDS	MW SIZING
Octyl glucoside	0–120 mM	• 2–40 kDA: Not compatible • 12–230 kDA: 0–60 mM 66-440 kDA: 0–60 mM	12–230 kDA: Slight decrease in signal at 120 mM	12–230 kDA: Slight decrease in resolution at 120 mM	• 12–230 kDA: Slight compression at 120 mM	12–230 kDA: May affect sizing due to compression

Detergents (for Fluorescence Detection Only)

COMPONENT	TEST RANGE	COMPATIBLE RANGE	FLUORESCENT SIGNAL	FLUORESCENT RESOLUTION	FLUORESCENT STANDARDS	MW SIZING	PROTEIN NORMALIZATION
Triton X–100	1%	0–0.5%	• 2–40 kDA:Severe peak broadening at ≥1% • 12–230 kDA: Signal loss ≥1% • 66–440 kDA: signal loss≥1%	• 2–40 kDA: Poor resolution at ≥1% • 12–230 kDA: resolution decrease	2–40 kDA: Peak splitting of 1 kDA standard at ≤0.25%	2–40 kDA: Compression of standards (1 kDA and 26 kDA run closer) at >0.5%	• 2–40 kDA: compression of standards causes PN curve to shift but no effect on signal. • 12–230 kDA: no effect • 66–40 kDA: no effect
C7BzO	1%	0–0.25%	• 2–40 kDA:Severe peak broadening at ≥1% • 12–230 kDA: Signal loss ≥1% • 66–440 kDA: signal loss≥1%	2–40 kDA: Poor resolution at ≥0.5%	• 2–40 kDA: Peak broadening for 1 kDA standard • 12–230 kDA: slight compression at 1%	Compression of standards (1 kDA and 26 kDA run closer) at ≥0.5%	• 2–40 kDA: compression of standards causes PN curve to shift but no effect on signal. • 12–230 kDA: no effect 6 • 6–40 kDA: no effect
CHAPS	2%	0.6–2%	No effect	No effect	No effect	12–230 kDA: Compression of standards (1 kDA and 26 kDA run closer) at 2%. Prepare biotinylated ladder in the same buffer for consistent sizing. Separation time may be increased to offset compression of standards.	No effect in 2–40 kDA, 12–23 kDA and 66–440 kDa
SDS	2%	1–2%	• 2–40 kDA: Peak broadening at 2% • 66–440 kDA: Signal decrease at 2%	Decreased resolution at 2%	• 2–40 kDA: Peak splitting of 1 kDA standard • 12–230 kDA: Std 1 kDA smear slightly	No effect	No effect in 2–40 kDA, 12–23 kDA and 66–440 kDa
Sodium deoxycholate	1%	0–1%	No effect	No effect	• 2–40 kDA: Peak splitting of 1 kDA standard at 1% • 12–230 kDA:Peak splitting of 1 kDA standard at 1%	No effect	No effect in 2–40 kDA, 12–23 kDA and 66–440 kDa
LDS	2%	0.5–1%	• 2–40 kDA: Peak broadening at 2% • 66–440 kDA: Signal decrease at 2%	Poor resolution at 2%	• 2–40 kDA: Peak splitting of 1 kDA standard at ≥1% 12–230: std 1 kDA sligtly smear • 66–440 kDA: std 230 kDA smearing	No effect	• 2–40 kDA: compression of standards causes PN curve to shift but no effect on signal. • 12–230 kDA: no effect • 66–40 kDA: no effect
Sarkosyl	1%	0–0.5%	2–40 kDA: Peak broadening at ≥1%	2–40 kDA: Poor resolution at ≥1%	• 2–40 kDA:Peak broadening for 1 kDA standard • 12–230 kDA:Peak broadening for 1 kDA standard	No effect	No effect in 2–40 kDA, 12–23 kDA and 66–440 kDa

Simple Western Size Assay Buffer Compatibility (Jess, Abby, Wes, Sally Sue, and Peggy Sue)

Fixative

COMPONENT	TEST RANGE	COMPATIBLE RANGE	CHEMI SIGNAL	CHEMI RESOLUTION	FLUORESCENT STANDARDS	MW SIZING
Formaldehyde	0–0.2%	0–0.1%	12–230 kDa: Signal decrease ≥0.1%	12–230 kDa: Resolution slightly decrease at ≥0.1%	12–230 kDa: Std 1 front end slightly smeared but picked up consistently >0.1%	No effect

Fixative (for Fluorescence Detection Only)

COMPONENT	TEST RANGE	COMPATIBLE RANGE	FLUORESCENT SIGNAL	FLUORESCENT RESOLUTION	FLUORESCENT STANDARDS	MW SIZING	PROTEIN NORMALIZATION
Formaldehyde	0–0.1%	0.10%	No effect	12-230 kDa: Slight resolution decrease	No effect	No effect	No effect

Viscosity/Density Additives

COMPONENT	TEST RANGE	COMPATIBLE RANGE	CHEMI SIGNAL	CHEMI RESOLUTION	FLUORESCENT STANDARDS	MW SIZING
Glycerol	0–20%	0–20%	No effect	• 12–230 kDa: Possible slight decrease in resolution in 0.02 mg/mL HeLa at 20%	12–230 kDa: Std 1 front end slightly smeared, but picked up consistently	No effect
Sucrose	0–300 mM	0–300 mM	No effect	No effect	12–230 kDa: Std 1 front end slightly smeared, but picked up consistently	No effect
PEG MW 20,000	0–5%	0–0.05%	• 2–40 kDa: Extreme peak broadening at ≥0.1% • 12–230 kDa: Signal loss ≥1% • 66-440kDa: Signal loss ≥1%	Resolution loss ≥1%	Complete resolution loss ≥1%	Affects sizing due to standards resolution loss. Affect minimized at ≤0.05%

Viscosity/Density Additives (for Fluorescence Detection Only)

COMPONENT	TEST RANGE	COMPATIBLE RANGE	FLUORESCENT SIGNAL	FLUORESCENT RESOLUTION	FLUORESCENT STANDARDS	MW SIZING	PROTEIN NORMALIZATION
Glycerol	20%	0–20%	No effect	No effect	No effect	No effect	No effect
Sucrose	300 mM	0–300 mM	No effect	No effect	No effect	No effect	No effect
PEG MW 20,000	1%	0–0.06%	• 2–40 kDa: Extreme peak broadening at ≥0.1%	Loss in resolution	• Extreme peak broadening of 1 kDa standard • 12–230 kDa: Peak splitting for 1 kDa std	Minimal effect at ≤0.06%	No effect

Miscellaneous Reagents

COMPONENT	TEST RANGE	COMPATIBLE RANGE	CHEMI SIGNAL	CHEMI RESOLUTION	FLUORESCENT STANDARDS	MW SIZING
EDTA	0–40 mM	0–40 mM	No effect	No effect	No effect	No effect
Imidazole	0–100 mM	• 2–40 kDa: 0–100 mM • 12–230 kDa: 0–50 mM • 66–440 kDa: 0–100 mM	• 12–230 kDa: Signal decrease at 100 mM especially for high MW targets	No effect	• 12–230 kDa: Std 1 front end slightly smeared, but picked up consistently	No effect

Miscellaneous Reagents (for Fluorescence Detection Only)

COMPONENT	TEST RANGE	COMPATIBLE RANGE	FLUORESCENT SIGNAL	FLUORESCENT RESOLUTION	FLUORESCENT STANDARDS	MW SIZING	PROTEIN NORMALIZATION
EDTA	40 mM	0–40 mM	2-40 kDa: Slight signal increase	No effect	No effect	No effect	No effect

Buffer

COMPONENT	TEST RANGE	COMPATIBLE RANGE	CHEMI SIGNAL	CHEMI RESOLUTION	FLUORESCENT STANDARDS	MW SIZING
PBS	50–90%	50%	• 12–230 kDa: Slight loss of signal at 90% • 66–440kDa: Slight loss of signal at 90%	• 12–230 kDa: Slight loss of resolution at 90%	• 12–230 kDa: Slight smearing of Std 230 kDa at 90% • 66–440 kDa: Slight smearing of Std 280 kDa at 90%	• 12–230 kDa: May affect sizing at HMW at 90%, due to smearing of Standard 230 kDa

Buffer (for Fluorescence Detection Only)

COMPONENT	TEST RANGE	COMPATIBLE RANGE	FLUORESCENT SIGNAL	FLUORESCENT RESOLUTION	FLUORESCENT STANDARDS	MW SIZING	PROTEIN NORMALIZATION
PBS	1X	0.25X–1X	66–440 kDa: Slight signal decrease	• 12–230 kDa: Slight loss of resolution	• 66–440kDa: Std 280 kDa smearing slightly	No effect	No effect

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