Buffer conditions

switchSENSE® measurements can be performed with many different buffer systems, e.g. **PBS**, **TRIS**, **HEPES**, **MOPS**, **MES**, …

The pH value may range from **pH 5 to pH 10**.

The salt concentration may range from **0.01 – 3 M** for static fluorescence measurements. For switching experiments, we recommend a monovalent salt concentration of **40 mM** for measurements with Adapter nanolevers and **140 mM** for measurements with Origami nanolevers.

For standard experiments, 10 mM buffer salt and 40/140 mM added monovalent salt (NaCl, KCl, …) is recommended. A minimum concentration of **10 mM** monovalent salt is required for all **switch**SENSE® measurements. DBS offers the following pH 7.4 buffers:

TE40	10 mM Tris-HCl	40 mM NaCl	0.05% Tween20	50 µM EDTA
PE40	10 mM Na_2HPO_4/NaH_2PO_4	40 mM NaCl	0.05% Tween20	50 µM EDTA
HE40	10 mM Hepes	40 mM NaCl	0.05% Tween20	50 µM EDTA
TE140	10 mM Tris-HCl	140 mM NaCl	0.05% Tween20	50 µM EDTA
PE140	10 mM Na_2HPO_4/NaH_2PO_4	140 mM NaCl	0.05% Tween20	50 µM EDTA
HE140	10 mM Hepes	140 mM NaCl	0.05% Tween20	50 µM EDTA

Considerations when preparing your own buffer:

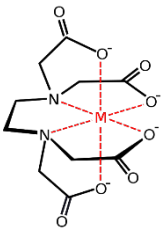
When performing measurements with *electrically actuated double stranded* DNA nanolevers, make sure that the cation concentration is high enough to maintain DNA duplex integrity ($[Na^+]$ or $[K^+] > 20$ mM). Divalent cations can also be added, e.g. $[Mg^{2+}] = 5$ mM, to further increase duplex stability.

Note: The addition of a **surfactant** (e.g. Tween) is required (std. = 0.05 % v/v).

EDTA

Ethylenediaminetetraacetic acid **0 – 20 mM**

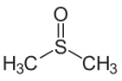
Scavenger for metal ions, $EDTA^{4-}$ chelates metal ions (e.g. Ni^{2+}, Ca^{2+}, Fe^{3+}), thereby reducing their activity in solution.



Scheme shows chelate complex with metal ion.
$C_{10}H_{16}N_2O_8$, MW 292 Da, solid, pK_a 1.782, density 860 g/l

DMSO

Dimethyl sulfoxide **0 – 5 % v/v**

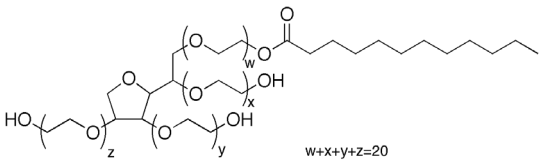


MW 78 Da, density 1.1 g/ml, viscosity 2.0 mPas at 20 °C (cf. $H_2O = 1$)

Dissolves polar and non-polar compounds, soluble in water and organic solvents, inhibits secondary structure formation in DNA (e.g. 10% used in PCR, lowers primer T_m by 5°C), also used as cryoprotectant.

Polysorbate 20 (Tween20®)

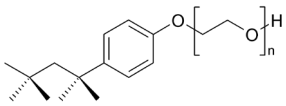
Non-ionic surfactant **0 – 1 % v/v, std. = 0.05 % v/v**



MW 1230 Da, density 1.1 g/ml, viscosity 250 – 400 mPas at 25°C (cf. H_2O = 1)

Triton® X-100

Non-ionic surfactant **0 – 1 % v/v, std. = 0.05 % v/v**



$C_{14}H_{22}O(C_2H_4O)_n$ (n = 9-10), MW 647 Da, density 1.07 g/ml, viscosity 240 mPas at 25°C (cf. H_2O = 1), melting point 6°C, 5% aq. solution = pH6.

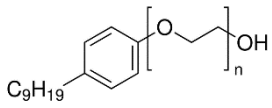
Soluble in water, miscible in most polar organic solvents & aromatic hydrocarbons, insoluble in aliphatic hydrocarbons, chemically stable in most acidic & alkaline solutions, compatible with anionic, cationic, and other nonionic surfactants

Nonidet® P40
Tergitol® NP-40

Non-ionic detergents / surfactants **0 – 1 % v/v, std. = 0.05 % v/v**

Nonidet P40, octyl phenoxypolyethoxyethanol
Tergitol® NP-40, nonyl phenoxypolyethoxylethanol

Nonidet® P40 substitute (from Sigma Aldrich)



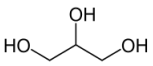
Average MW 680 Da
pH 5-8 (5% in H_2O)
CMC 0.059 mM (20-25°C)

Used for the extraction of proteins from (eukaryotic) cells, for instance in the lysis buffer RIPA (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS), or to solubilize GABA receptors.

Can enhance *or* suppress interactions between DNA and proteins, often used to reduce artefacts like non-specific binding of transcription factors.

Glycerol

Stabilizer and viscous co-solvent **0 – 20 wt%**



$C_3H_8O_3$, MW 92 Da, liquid, density 1.26 g/ml,

viscosity 1412 mPas (cf. H_2O = 1), melting point 17.8°C

Kosmotrope, causes water molecules to favorably interact, thereby stabilizing intramolecular interactions in proteins. Antifreeze, hygroscopic.

Can be used to increase the solution viscosity. Does not impair switching efficiency, but slows switching dynamics.

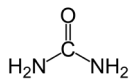
Viscosities of glycerol-water mixtures at 20°C

Glycerol (wt%)	0	10	20	30	40	50	60	65	70	75	80
Viscosity (mPas)	1.005	1.31	1.76	2.50	3.72	6.00	10.8	15.2	22.5	35.5	60.1

[from "Properties of ordinary water-substance" NE Dorsey p.184, New York (1940)]

Urea

Denatures proteins by disrupting non-covalent bonds **0 – 5 M**



CH_4N_2O, MW 60 Da, density 1.32 g/cm³, melting point 133°C, solid, soluble in water >1g/ml at 20°C.

Increases solution viscosity, glycerol can be used for reference measurements.

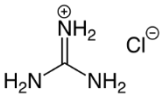
Viscosities of urea-water mixtures at 25°C

Urea (wt%)	6.16	10.57	13.92	18.31	22.66	25.54	31.02	33.11	35.07	39.10	43.1
Urea (mol/l)	1.039	1.804	2.399	3.191	3.994	4.537	5.590	5.999	6.388	7.198	8.019
Viscosity (mPas)	1.043	1.080	1.113	1.162	1.219	1.265	1.359	1.403	1.449	1.545	1.663

[Kawahara K, Tanford C, *J. Biol. Chem.* (1966)241:3228]

Guanidine hydrochloride

Denatures proteins by disrupting non-covalent bonds increases solution ionic strength **0 – 6 M** (GuHCl)



CH_6ClN_3, solid, MW 95.5 Da, density 1.354 g/cm³, melting point 182°C

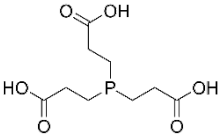
Very good solubility in water and ethanol. Increases solution viscosity. Very strong denaturation action, most proteins lose their structure in 6M GuHCl.

Note that due to the ionic nature of the guanidium cation and chloride anion, an electrical actuation of **switch**SENSE® layers may not be possible at high concentrations (>0.1 M).

TCEP

Tris(2-carboxyethyl)phosphine Reducing agent **0 – 1 mM**

Reducing agent



$C_9H_{15}O_6P$, MW 250.2 Da, often used as hydrochloride salt (TCEP-HCl, MW 286.7 Da), odorless, very good water solubility.

Strong reducing agent. Breaks disulfide bonds within proteins, for instance useful when labeling cysteine residues with maleimides.

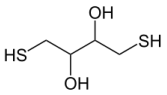
If used as hydrochloric salt (TCEP-HCl), be careful not to acidify the solution (use appropriate buffer capacity).

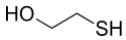
DTT, BME

Dithiothreitol and β-mercaptoethanol **0 M**

Reducing agents

Note: Please replace DTT or BME with TCEP



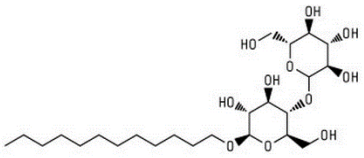


The reducing agents Dithiothreitol (DTT, *left*) and β-mercaptoethanol (BME, *right*) cause a reductive desorption of **switch**SENSE® layers. Proteins with DTT need to be not only replaced with TCEP but also washed 5 times with buffer.

DDM

n-Dodecyl-beta-D-maltoside **0 – 20+ mM**

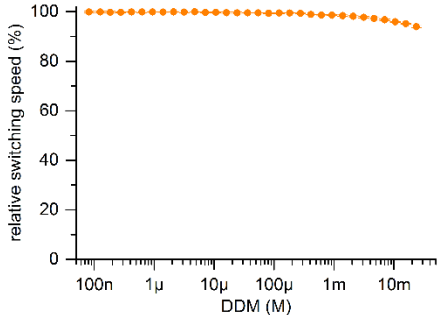
Non-ionic detergent



$C_{24}H_{46}O_{11}$, MW 510.6 Da, Micelle MW 50,000 Da

pH (1% Solution): 5 to 8; Solubility (in water at 0 to 5°C): ≥20%; Conductivity (10% Solution) : <40mS

Critical Micelle Concentration (CMC): 0.17mM (0.009%, w/v) in water ; 0.12mM (0.006%, w/v) in 0.2M NaCl

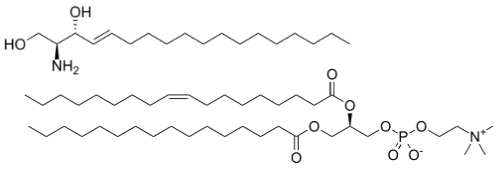


A measurable decrease in switching dynamics (increase in solution viscosity) may occur when used at high concentrations (100xCMC), but this does not impair the measurement.

Lipids

Sphingolipids and phospholipids **up to several mM**

Various sphingolipids and phospholipids can be added to the running buffer.



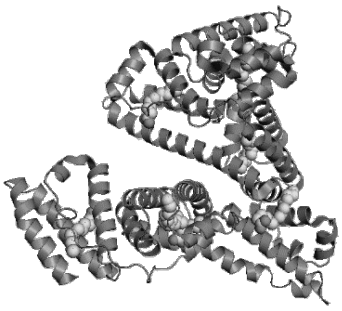
D-Sphingosine

POPC (in eukaryotic cell membranes, 2-Oleoyl-1-palmitoyl-sn-glycero-3-phosphocholine)

L-α-lecithin (L- α -Phosphatidylcholine) (no image)

A measurable decrease in switching dynamics (increase in solution viscosity) may occur when used at high concentrations, but this does not impair the measurement.

Serum Albumin	**Human Serum Albumin (HSA)**	**< 0.1% v/v**
	Bovine Serum Albumin (BSA)	**< 0.1% v/v**



HSA (MW 67 kDa, PDB 1e7h) is the most abundant protein in blood plasma at concentrations between 35 – 50 g/l (0.5 – 0.75 mM); it transports hormones, fatty acids, and other compounds, buffers pH, and maintains osmotic pressure. HSA and BSA are particularly "sticky" proteins which are prone to non-specific adsorption on walls of vessels and tubing. Therefore, they are often used for passivating walls against non-specific adsorption of other proteins.

switchSENSE® measurements can be carried out at HSA and BSA concentrations below 0.1%. Note, however, that high albumin concentrations lead to measurable changes in the solution viscosity (slower switching dynamics) and increase the background fluorescence due to autofluorescence of solute protein in the flow channel, which can be accounted for by reference subtraction.

Cell lysate

switchSENSE® measurements can be performed in various cell lysate solutions and supernatants. The addition of EDTA to inhibit DNase activity may be advised. Clean the device intensively after the measurement. Please contact support@dynamic-biosensors.com for more information.

Didn't find the info you were interested in?
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