

### switchSENSE® compatibility sheet

# Buffer conditions

switch SENSE® 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 1 - 300 mM for switching experiments and 0 - 3 M for fluorescence measurements.

For standard experiments, 10 mM buffer salt and 40 mM added monovalent salt (NaCl, KCl, ...) is recommended. DBS offers the following pH 7.4 buffers:

T40 (TE40)	10 mM Tris-HCl	40 mM NaCl	0.05% Tween20	(5 mM EDTA)
T20/5	10 mM Tris-HCl	20 mM NaCl	5 mM MgCl <sub>2</sub>	
P40 (PE40)	10 mM Na <sub>2</sub> HPO <sub>4</sub> /NaH <sub>2</sub> PO <sub>4</sub>	40 mM NaCl	0.05% Tween20	(5 mM EDTA)
H40 (HE40)	10 mM Hepes	40 mM NaCl	0.05% Tween20	(5 mM EDTA)

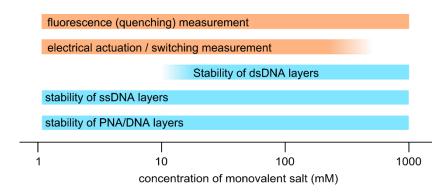
Considerations when preparing your own buffer:

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. When using single stranded DNA or PNA/DNA hybrids, very low salinity (1 mM and lower) can be used. The addition of a surfactant (e.g. Tween) is recommended.

For measurements that involve the electrical actuation of DNA nanolevers (DNA switching), the concentration of monovalent salt should be in the range from **1 to 300 mM** (in solutions of high ionic strength electric fields are screened, which attenuates electrical interactions between the electrodes and the DNA nanolevers).

Fluorescence measurements can be performed at salt concentrations from **0 to 3 M**.

Guideline for the concentration ranges of monovalent salts in solution



### **Temperature**

### 96 bp DNA nanolevers:

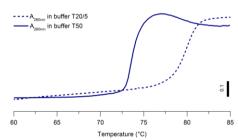
**4 – 80°C** in 10 mM Tris buffer, [Na<sup>+</sup>] or [K<sup>+</sup>] ≥ 20mM and [Mg<sup>2+</sup>] = 5mM



#### 48 bp DNA nanolevers:

**4 – 75°C** in 10 mM Tris buffer, [Na<sup>+</sup>] or [K<sup>+</sup>] ≥ 20mM and [Mg<sup>2+</sup>] = 5mM

**4 – 70°C** in 10 mM Tris buffer,  $[Na^+]$  or  $[K^+]$  ≥ 50mM (pH 7.4)



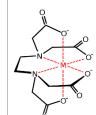
Melting analysis of standard dsDNA nanolevers in solution by DNA hypochromicity

# **EDTA ●○○**

Ethylenediaminetetraacetic acid

0-5 mM

Scavenger for metal ions, EDTA<sup>4-</sup> chelates metal ions (e.g. Ni<sup>2+,</sup> Ca<sup>2+</sup>, Fe<sup>3+</sup>), 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<sub>a</sub> 1.782, density 860 g/l

## **DMSO**

Dimethyl sulfoxide

0 - 5 % v/v

$$_{\parallel}^{O}$$
 MW 78 Da, density 1.1 g/ml,  
 $_{H_3C}^{S}$  CH<sub>3</sub> 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

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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

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 $C_{14}H_{22}O(C_2H_4O)_n$  (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.



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	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  O	Non-ionic detergents / surfactants $0-1\% \text{ v/v}$ , std. = $0.05\% \text{ v/v}$ 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 <sub>2</sub> O)  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 <i>or</i> 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 – 70 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	[Holli Troperdes of Ordinary Water Substance Ne Dolsey p. 104, New York (1540)]					
Urea	Denatures proteins by disrupting non-covalent bonds  0 – 8 M					
	Denatures proteins by disrupting non-covalent bonds $0-8 \text{ M}$ $\bigcirc$ CH <sub>4</sub> N <sub>2</sub> O, MW 60 Da, density 1.32 g/cm <sup>3</sup> , melting point 133°C,					
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Guanidine
hydrochloride
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Denatures proteins by disrupting non-covalent bonds (GuHCl) increa

0 – 6 M

increases solution ionic strength

CH<sub>6</sub>ClN<sub>3</sub>, solid, MW 95.5 Da, density 1.354 g/cm<sup>3</sup>, melting point 182°C

Very good solubility in water and ethanol. Increases solution viscosity, glycerol can be used for reference measurements. 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 <a href="mailto:switchSENSE">switchSENSE</a>® layers may not be possible at high concentrations (>0.1M).

## TCEP O

Reducing agent tris(2-carboxyethyl)phosphine

0 – 1 mM

electrical actuation 0 - 0.1 mM

 $C_9H_{15}O_6P$ , 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-HCI), be careful not to acidify the solution (use appropriate buffer capacity).

# DTT β-mercapto-ethanol

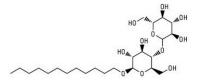
**Reducing agents** 

 $$<\!10~\mu M$  when switching mM concentrations at negative DC potentials

Reducing agents Dithiothreitol (DTT, left) and  $\beta$ -mercaptoethanol ( $\beta$ MCE, right) may cause a reductive desorption of **switch**SENSE® layers at high concentrations. Reduce the DTT concentration prior to experiment or consider alternatives such as TCEP. Note, association measurement without the use of dynamic measurements and dissociation measurements are unaffected by the use of DTT. For more advice, contact <a href="info@dynamic-biosensors.com">info@dynamic-biosensors.com</a>

### DDM OO

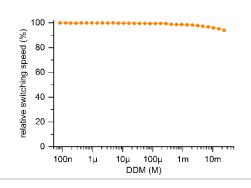
Non-ionic detergent n-Dodecyl-beta-D-maltoside 0 - 20 + mM



 $C_{24}H_{46}O_{11}$ , MW 510.6 Da, Micelle MW 50,000 Da pH (1% Solution): 5 to 8; Solubility (in water at 0 to 5°C):  $\geq$ 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 OO

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- $\alpha$ -lecithin (L- $\alpha$  -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) Bovine Serum Albumin (BSA) 0 – 0.5 mM (3.5%) normal serum conc.



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.

switch SENSE® measurements can be carried out at high HSA concentrations corresponding to normal serum levels (0.5 mM). 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. If not required otherwise, HSA concentrations <0.1mM (1%) are advised.



Cell lysate

switch SENSE\* measurements can be performed in various cell lysate solutions and supernatants. The addition of EDTA to inhibit DNase activity may be advised. Please contact <a href="mailto:info@dynamic-biosensors.com">info@dynamic-biosensors.com</a> for more information.

Didn't find the info you were interested in? Please contact <a href="mailto:info@dynamic-biosensors.com">info@dynamic-biosensors.com</a>.