NANOTEMPER

Buffer Exploration Kit

Assay Development Kit –
Systematic Buffer Optimisation for Interaction Screening

Cat# NT-B001

produced by
Jena Bioscience

Content

1. General information

1.1. Content and storage

1x Deep-well plate with 96 different buffer conditions (details listed below), 1 ml each.

The Buffer Exploration Kit is shipped with cool packs and should be stored at -20 °C upon arrival. The product's shelf life can be found on the kit box. Note that it is recommended to re-seal the kit with conventional sealing foil after opening and we generally advise to not use the kit more than 3 months after opening. Each kit contains 1 ml of all buffer reagents, enough for at least 12 buffer screenings performed using the protocol outlined in this document.

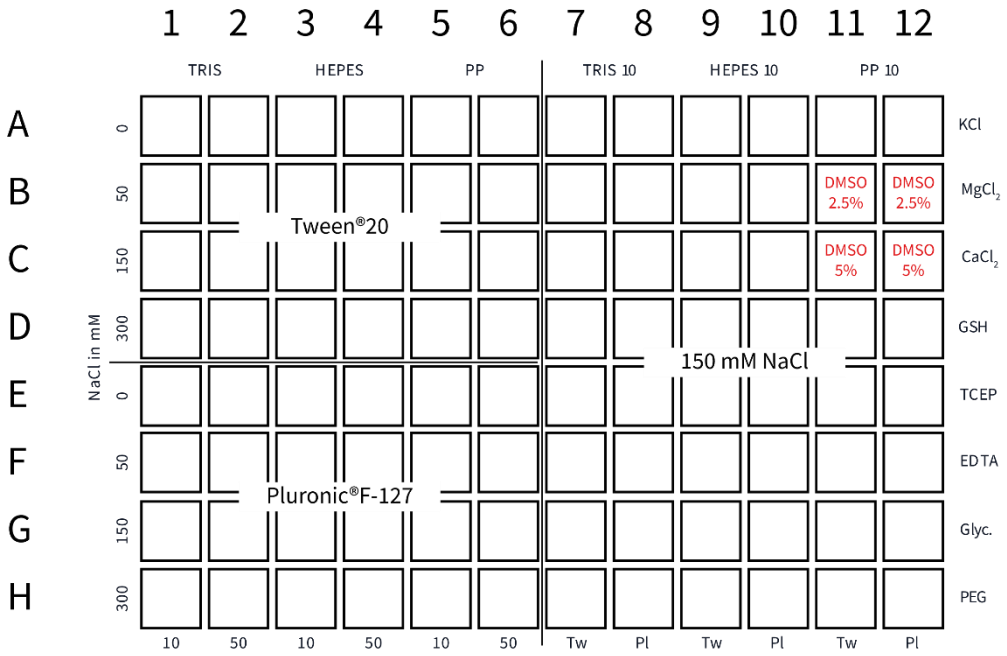
1.2. Introduction to applications

The Buffer Exploration Kit contains a deep-well plate with 96 different buffer conditions for systematic, plate-based assay development. The layout enables a fully automated workflow using standard robotic liquid handlers.

The kit is recommended

- when assay conditions are established for the first time
- when facing target stability issues of an already established interaction
- to avoid sample quality issues such as target aggregation

1.3. Overview



PP: Na_2HPO_4 + KH_2PO_4
Tween20®: 0.005 %
Pluronic® F-127: 0.1 %
KCl: 3 mM
$MgCl_2$: 10 mM
$CaCl_2$: 10mM
GSH: 2 mM
TCEP: 1 mM
EDTA: 5 mM
Glycerol: 10 %
PEG 8000: 1 g/l
DMSO: Only in PP buffer instead of $CaCl_2$ or $MgCl_2$

2. Screen Formulation

Table 1: Buffer Exploration Kit formulation. A Microsoft Excel file can be downloaded from the NanoTemper Technologies explorer community (https://nanotempertech.com/be-an-explorer/).

Well ID	Buffer	c (Buffer) [mM]	Buffer pH	c (NaCl) [mM]	Detergent	c (Detergent) [% w/v]	Additive	c (Additive)
A1	TRIS	10	7.40	0	Tween®-20	0.005	-	-
A2	TRIS	50	7.40	0	Tween®-20	0.005	-	-
A3	HEPES	10	7.40	0	Tween®-20	0.005	-	-
A4	HEPES	50	7.40	0	Tween®-20	0.005	-	-
A5	Na_2HPO_4/KH_2PO_4	10	7.40	0	Tween®-20	0.005	-	-
A6	Na_2HPO_4/KH_2PO_4	50	7.40	0	Tween®-20	0.005	-	-
A7	TRIS	10	7.40	150	Tween®-20	0.005	KCl	3 mM
A8	TRIS	10	7.40	150	Pluronic®F-127	0.1	KCl	3 mM
A9	HEPES	10	7.40	150	Tween®-20	0.005	KCl	3 mM
A10	HEPES	10	7.40	150	Pluronic®F-127	0.1	KCl	3 mM
A11	Na_2HPO_4/KH_2PO_4	10	7.40	150	Tween®-20	0.005	KCl	3 mM
A12	Na_2HPO_4/KH_2PO_4	10	7.40	150	Pluronic®F-127	0.1	KCl	3 mM
B1	TRIS	10	7.40	50	Tween®-20	0.005	-	-
B2	TRIS	50	7.40	50	Tween®-20	0.005	-	-
B3	HEPES	10	7.40	50	Tween®-20	0.005	-	-
B4	HEPES	50	7.40	50	Tween®-20	0.005	-	-
B5	Na_2HPO_4/KH_2PO_4	10	7.40	50	Tween®-20	0.005	-	-
B6	Na_2HPO_4/KH_2PO_4	50	7.40	50	Tween®-20	0.005	-	-
B7	TRIS	10	7.40	150	Tween®-20	0.005	$MgCl_2$	10 mM
B8	TRIS	10	7.40	150	Pluronic®F-127	0.1	$MgCl_2$	10 mM
B9	HEPES	10	7.40	150	Tween®-20	0.005	$MgCl_2$	10 mM
B10	HEPES	10	7.40	150	Pluronic®F-127	0.1	$MgCl_2$	10 mM
B11	Na_2HPO_4/KH_2PO_4	10	7.40	150	Tween®-20	0.005	DMSO	2.5 % v/v
B12	Na_2HPO_4/KH_2PO_4	10	7.40	150	Pluronic®F-127	0.1	DMSO	2.5 % v/v
C1	TRIS	10	7.40	150	Tween®-20	0.005	-	-
C2	TRIS	50	7.40	150	Tween®-20	0.005	-	-
C3	HEPES	10	7.40	150	Tween®-20	0.005	-	-
C4	HEPES	50	7.40	150	Tween®-20	0.005	-	-
C5	Na_2HPO_4/KH_2PO_4	10	7.40	150	Tween®-20	0.005	-	-
C6	Na_2HPO_4/KH_2PO_4	50	7.40	150	Tween®-20	0.005	-	-
C7	TRIS	10	7.40	150	Tween®-20	0.005	$CaCl_2$	10 mM
C8	TRIS	10	7.40	150	Pluronic®F-127	0.1	$CaCl_2$	10 mM
C9	HEPES	10	7.40	150	Tween®-20	0.005	$CaCl_2$	10 mM
C10	HEPES	10	7.40	150	Pluronic®F-127	0.1	$CaCl_2$	10 mM
C11	Na_2HPO_4/KH_2PO_4	10	7.40	150	Tween®-20	0.005	DMSO	5 % v/v
C12	Na_2HPO_4/KH_2PO_4	10	7.40	150	Pluronic®F-127	0.1	DMSO	5 % v/v
D1	TRIS	10	7.40	300	Tween®-20	0.005	-	-
D2	TRIS	50	7.40	300	Tween®-20	0.005	-	-
D3	HEPES	10	7.40	300	Tween®-20	0.005	-	-
D4	HEPES	50	7.40	300	Tween®-20	0.005	-	-
D5	Na_2HPO_4/KH_2PO_4	10	7.40	300	Tween®-20	0.005	-	-
D6	Na_2HPO_4/KH_2PO_4	50	7.40	300	Tween®-20	0.005	-	-
D7	TRIS	10	7.40	150	Tween®-20	0.005	GSH	2 mM
D8	TRIS	10	7.40	150	Pluronic®F-127	0.1	GSH	2 mM
D9	HEPES	10	7.40	150	Tween®-20	0.005	GSH	2 mM
D10	HEPES	10	7.40	150	Pluronic®F-127	0.1	GSH	2 mM
D11	Na_2HPO_4/KH_2PO_4	10	7.40	150	Tween®-20	0.005	GSH	2 mM
D12	Na_2HPO_4/KH_2PO_4	10	7.40	150	Pluronic®F-127	0.1	GSH	2 mM

Well ID	Buffer	c (Buffer) [mM]	Buffer pH	c (NaCl) [mM]	Detergent	c (Detergent) [% w/v]	Additive	c (Additive)
E1	TRIS	10	7.40	0	Pluronic®F-127	0.1	-	-
E2	TRIS	50	7.40	0	Pluronic®F-127	0.1	-	-
E3	HEPES	10	7.40	0	Pluronic®F-127	0.1	-	-
E4	HEPES	50	7.40	0	Pluronic®F-127	0.1	-	-
E5	Na_2HPO_4/KH_2PO_4	10	7.40	0	Pluronic®F-127	0.1	-	-
E6	Na_2HPO_4/KH_2PO_4	50	7.40	0	Pluronic®F-127	0.1	-	-
E7	TRIS	10	7.40	150	Tween®-20	0.005	TCEP	1 mM
E8	TRIS	10	7.40	150	Pluronic®F-127	0.1	TCEP	1 mM
E9	HEPES	10	7.40	150	Tween®-20	0.005	TCEP	1 mM
E10	HEPES	10	7.40	150	Pluronic®F-127	0.1	TCEP	1 mM
E11	Na_2HPO_4/KH_2PO_4	10	7.40	150	Tween®-20	0.005	TCEP	1 mM
E12	Na_2HPO_4/KH_2PO_4	10	7.40	150	Pluronic®F-127	0.1	TCEP	1 mM
F1	TRIS	10	7.40	50	Pluronic®F-127	0.1	-	-
F2	TRIS	50	7.40	50	Pluronic®F-127	0.1	-	-
F3	HEPES	10	7.40	50	Pluronic®F-127	0.1	-	-
F4	HEPES	50	7.40	50	Pluronic®F-127	0.1	-	-
F5	Na_2HPO_4/KH_2PO_4	10	7.40	50	Pluronic®F-127	0.1	-	-
F6	Na_2HPO_4/KH_2PO_4	50	7.40	50	Pluronic®F-127	0.1	-	-
F7	TRIS	10	7.40	150	Tween®-20	0.005	EDTA	5 mM
F8	TRIS	10	7.40	150	Pluronic®F-127	0.1	EDTA	5 mM
F9	HEPES	10	7.40	150	Tween®-20	0.005	EDTA	5 mM
F10	HEPES	10	7.40	150	Pluronic®F-127	0.1	EDTA	5 mM
F11	Na_2HPO_4/KH_2PO_4	10	7.40	150	Tween®-20	0.005	EDTA	5 mM
F12	Na_2HPO_4/KH_2PO_4	10	7.40	150	Pluronic®F-127	0.1	EDTA	5 mM
G1	TRIS	10	7.40	150	Pluronic®F-127	0.1	-	-
G2	TRIS	50	7.40	150	Pluronic®F-127	0.1	-	-
G3	HEPES	10	7.40	150	Pluronic®F-127	0.1	-	-
G4	HEPES	50	7.40	150	Pluronic®F-127	0.1	-	-
G5	Na_2HPO_4/KH_2PO_4	10	7.40	150	Pluronic®F-127	0.1	-	-
G6	Na_2HPO_4/KH_2PO_4	50	7.40	150	Pluronic®F-127	0.1	-	-
G7	TRIS	10	7.40	150	Tween®-20	0.005	Glycerol	10 % v/v
G8	TRIS	10	7.40	150	Pluronic®F-127	0.1	Glycerol	10 % v/v
G9	HEPES	10	7.40	150	Tween®-20	0.005	Glycerol	10 % v/v
G10	HEPES	10	7.40	150	Pluronic®F-127	0.1	Glycerol	10 % v/v
G11	Na_2HPO_4/KH_2PO_4	10	7.40	150	Tween®-20	0.005	Glycerol	10 % v/v
G12	Na_2HPO_4/KH_2PO_4	10	7.40	150	Pluronic®F-127	0.1	Glycerol	10 % v/v
H1	TRIS	10	7.40	300	Pluronic®F-127	0.1	-	-
H2	TRIS	50	7.40	300	Pluronic®F-127	0.1	-	-
H3	HEPES	10	7.40	300	Pluronic®F-127	0.1	-	-
H4	HEPES	50	7.40	300	Pluronic®F-127	0.1	-	-
H5	Na_2HPO_4/KH_2PO_4	10	7.40	300	Pluronic®F-127	0.1	-	-
H6	Na_2HPO_4/KH_2PO_4	50	7.40	300	Pluronic®F-127	0.1	-	-
H7	TRIS	10	7.40	150	Tween®-20	0.005	PEG 8000	1 g/l
H8	TRIS	10	7.40	150	Pluronic®F-127	0.1	PEG 8000	1 g/l
H9	HEPES	10	7.40	150	Tween®-20	0.005	PEG 8000	1 g/l
H10	HEPES	10	7.40	150	Pluronic®F-127	0.1	PEG 8000	1 g/l
H11	Na_2HPO_4/KH_2PO_4	10	7.40	150	Tween®-20	0.005	PEG 8000	1 g/l
H12	Na_2HPO_4/KH_2PO_4	10	7.40	150	Pluronic®F-127	0.1	PEG 8000	1 g/l

* pH values indicated refer to the 1.0 M buffer stock solution prior to dilution with other components

3. General recommendations for sample preparation

An application example for the Buffer Exploration Kit is described in the NanoTemper Technologies technical note “Fast-track your hit screening assay development with the Buffer Exploration Kit”, which can be obtained from the NanoTemper Technologies Explorer Community (https://nanotempertech.com/be-an-explorer/).

For optimal performance of the kit and best possible results, please note the following general instructions.

- Do not use the plate for more than 3 months after breaking the seal.
- When using the kit after storage at -20 °C, make sure that all buffers have thawed completely before using it. Thawing should be done at room temperature.
- When storing the plate at -20 °C we recommend re-sealing a once opened plate with conventional glue sealing foil.
- At least 1:10 dilution of the initial sample buffer with the final buffer condition is advised to avoid bias from buffer components, originating from the initial sample buffer.
- Data evaluation should always consider the composition of the initial sample buffer. Although the advised 1:10 dilution into the final buffer solutions should sufficiently reduce the bias of the initial sample buffer, highly concentrated components can still have an impact on the obtained results. This should always be considered when formulating the selected buffer to reproduce results from a buffer screening with this kit.
- Thorough mixing is important. Check the mixing properties of your liquid handling system of choice and increase mixing cycles and/or mixing volume if necessary. We recommend at least 15 mixing cycles with 80% of the final volume.
- Repetitive measurements of buffer conditions at 30 min intervals over a period of 2-3 h can give additional information about stability of the tested sample in different buffers.
- Selection of buffer condition can influence molecular affinities (i.e. K_d, or EC50 values) e.g. by variation of ionic strength when binding is mediated by electrostatic interactions.
- TCEP can quench fluorescent dyes.
- Compatibility with instruments that measure UV fluorescence: Consider that Tween®20 can exhibit UV fluorescence, thus increasing the background fluorescence. In that case preferentially use conditions with Pluronic®F-127.

4. Protocols and recommended assay optimization strategies

4.1. General considerations

- Compatibility with NanoTemper Technologies His-Tag labelling kits: This kit is only partially suitable for NanoTemper Technologies His-Tag labelling kits (Cat# L008, L018, L118). If His-Tag labelling is used, the following buffer conditions should be excluded from evaluation: A1, A2, A7, A8, B1, B2, B7, B8, C1, C2, C7, C8, D1, D2, D7, D8, E1, E2, E7, E8, E9, E10, E11, E12, F1, F2, F7, F8, F9, F10, F11, F12, G1, G2, G7, G8, H1, H2, H7, H8.
- During incubation and measurement, we recommend sealing the Dianthus 384-well plate to avoid evaporation. Please note that seal should be removed before necessary centrifugation steps to remove air bubbles.

4.2. Protocol for use with Dianthus instruments in a 384-well layout

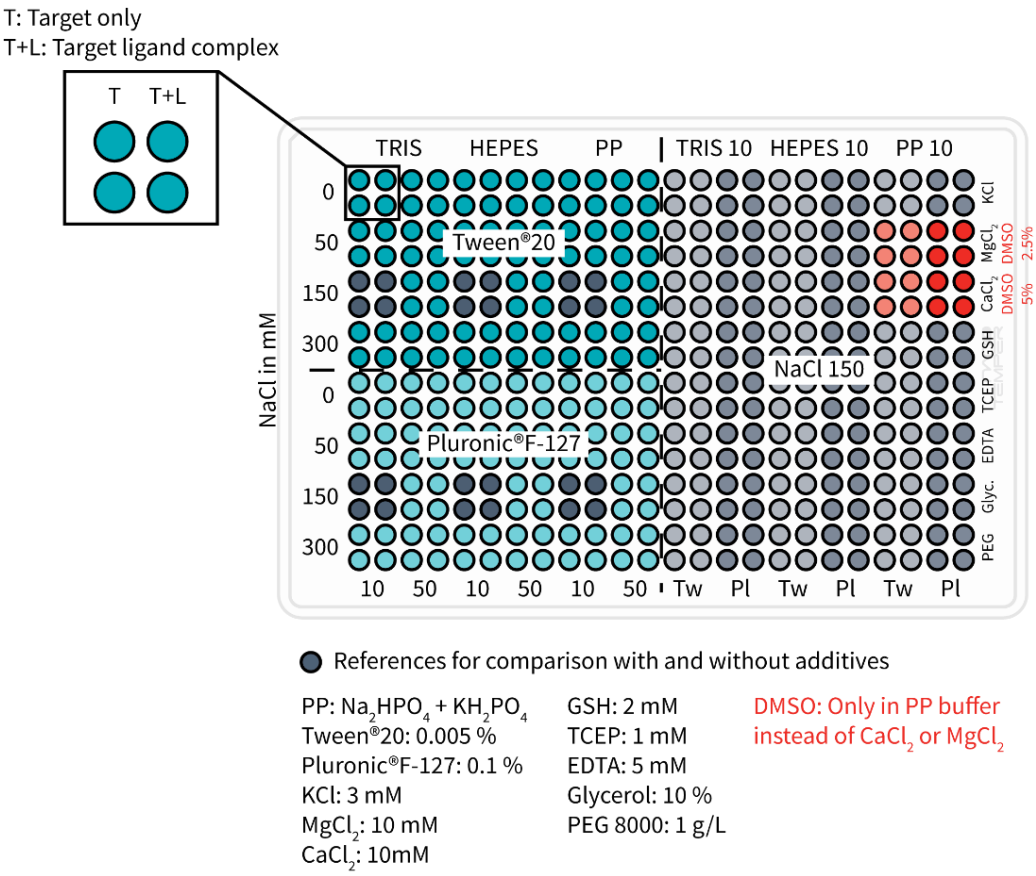
This protocol describes the standard procedure for the buffer screening step during the assay development for interaction screening with Dianthus instruments. In a nutshell 96 binding check experiments (signal comparison for the labelled target molecule in presence and absence of a saturating ligand concentration) in different buffer compositions will be prepared, measured and analysed. An application example for the Buffer Exploration Kit is described in the NanoTemper Technologies technical note “Fast-track your hit screening assay development with the Buffer Exploration Kit”, which can be obtained from the NanoTemper Technologies Explorer Community (https://nanotempertech.com/be-an-explorer/).

STEP1: Prepare two stock solutions, one for the reference sample (labelled target molecule only) and one for the positive control (labelled target molecule + ligand molecule at a saturating concentration) in a buffer of your choice. The reference and positive control stock solutions should be prepared as 10-fold concentrated stock solutions of the final assay concentration. For the assay a volume of each stock of at least 384 µl is needed. Please also account for potential dead volume needed for your liquid handling system of choice. We generally recommend preparing at least 580 µl of each 10-fold stock solution.

STEP2: Transfer 4 x 18 µl of each buffer solution from the 96-deep-well plate into a Dianthus 384-well plate. So, for each buffer 4 wells neighbouring in a square are filled with 18 µl each.

STEP3: Add 2 µl of the 10x reference stock to each well of the odd columns of the prefilled 384-well plate and mix them thoroughly. The wells of the even columns should be complemented in the same way with 2 µl of the 10x positive control stock.

Final layout of the Dianthus 384-well plate prefilled with 96 different buffer conditions from the Buffer Exploration Kit:



STEP4: Equilibrate the buffer screening assay for 0.5-2.5 hrs at room temperature in the dark (depending on the interaction) and then centrifuge the plate for 30 sec at 1000 x g before loading into a Dianthus NT.23 instrument.

STEP5: For measurement and evaluation open **DI.Control** software and choose the Buffer Screen experiment in the Assay Development section of the available experiment templates shown on the start screen.

4.3. Protocol for manual use with a selection of buffers (Monolith and Dianthus instruments)

The following protocol describes how the Buffer Exploration Kit can be used manually with any analytical method of choice. It was specially developed for manual use with a Monolith NT.115 or Monolith NT.Automated instrument but can also be applied to Dianthus instruments. It proceeds in two or three steps and tests a total of 14 to 17 different conditions.

STEP1: This step is meant to explore buffer substances and detergents. Prepare 6 binding check experiments using the following buffer solutions (well ID) from the left side of the plate.

C1, G1, C3, G3, C5, G5

The preparation of the binding check experiments should be done in the following way. Prepare two stock solutions, one for the reference sample (i.e. target molecule only) and one for the positive control (i.e. target molecule + ligand molecule at a saturating concentration) in a buffer of your choice. The reference and positive control stock solutions should be prepared as 10-fold concentrated stock solutions of the final assay concentration. Transfer 18 µl of each buffer solution from the 96-deep-well plate into a tube/well.

The NanoTemper Technologies Software solutions MO.Control and DI.Control have integrated binding check experiments. The number of replicates can be chosen freely in DI.Control (we recommend using the Binding Check experiment template or alternatively the Single Dose - Quick Start experiment. In MO.Control generally 4 replicates for reference and positive control each are needed for a binding check experiment, this number can be reduced by using the expert mode.

Add 2 µl of the 10x reference stock to each replicate tube/well for the reference and 2 µl positive control stock to each replicate/tube for the positive control and mix thoroughly.

STEP2: This step is meant to explore buffer additives. Based on the outcome of the experiment in STEP1, select the buffer, which delivered the best result (Signal to Noise Ratio/Signal Quality). Now with this buffer in mind in a second step the effect of buffer additives can be tested.

To test the impact of the different additives, prepare 8 more binding check experiments using the buffer solutions from the following columns of the right side of the plate, depending on the buffer you selected from STEP1. For preparation of the experiment proceed as described in STEP1.

Buffer from STEP1	Column to test in STEP2
C1	Column 7
G1	Column 8
C3	Column 9
G3	Column 10
C5	Column 11
G5	Column 12

Compare results from STEP2 with the result from STEP1. Did the Signal to Noise Ratio or the signal Quality improve for any of the conditions tested in STEP2? The row ID of the respective condition reveals which additive led to this improvement.

Row ID for improved condition	Additive
A	KCl (3 mM)
B	MgCl2 (10 mM) or DMSO (only PP buffer 2.5%)
C	CalCl2 (10 mM) or DMSO (only PP buffer 5%)
D	GSH (2 mM)
E	TCEP (1 mM)
F	EDTA (5 mM)
G	Glycerol (10 %)
H	PEG 8000 (1 g/l)

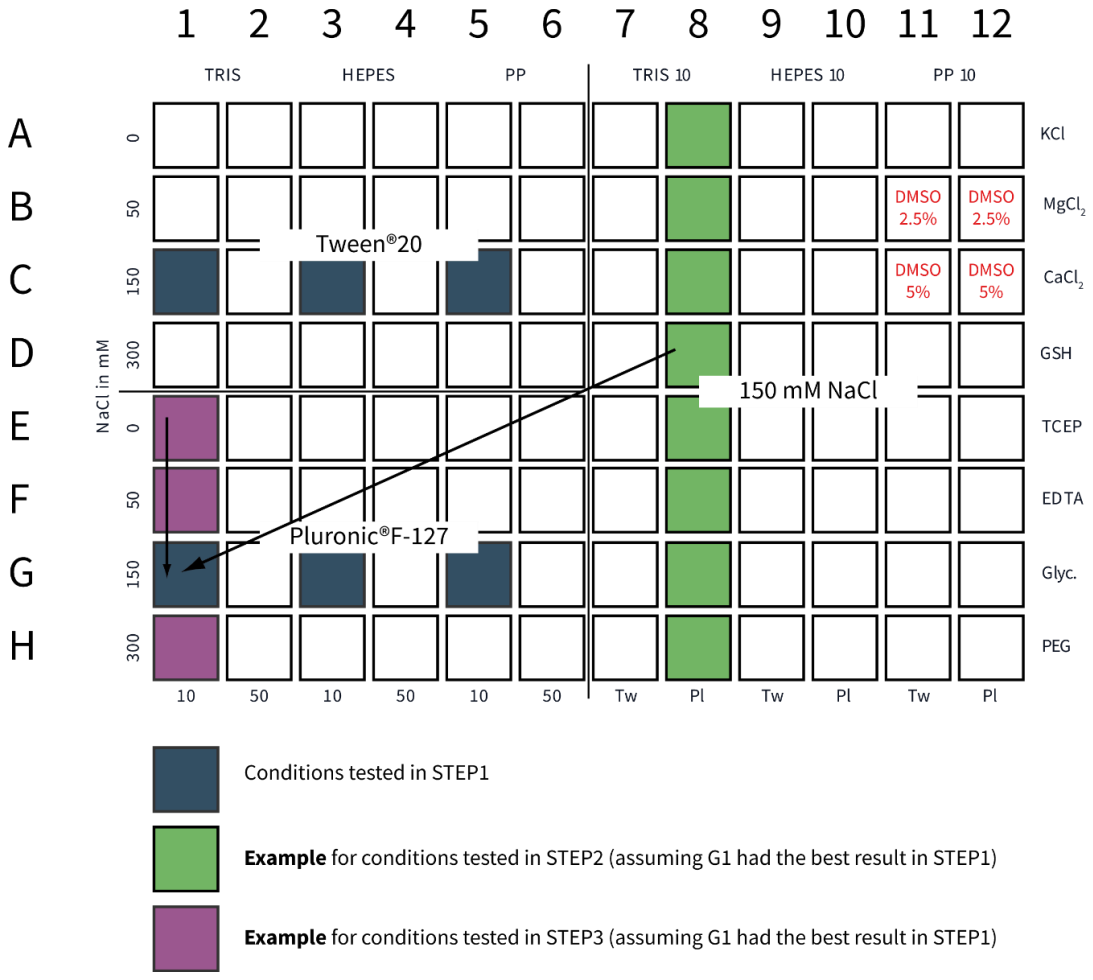
STEP3 (optional) This step is meant to explore ionic strength of the buffer. If you haven't noticed any improvement or even a deterioration of Signal Quality for STEP2, you can also vary the NaCl concentration to test the influence of the ionic strength of the buffer. For this purpose, choose the following conditions, depending on the buffer you selected in STEP1, and prepare 3 more binding check experiments.

Buffer from STEP1	Wells to test in STEP2
C1	A1, B1, D1
G1	E1, F1, H1
C3	A3, B3, D3
G3	E3, F3, H3
C5	A5, B5, D5
G5	E5, F5, H5

Compare results from STEP3 with the result of the buffer selected in STEP1. Did the Signal to Noise Ratio or the Signal Quality improve for any of the conditions tested in STEP3? The row ID of the respective condition reveals which NaCl concentration led to this improvement.

Row ID for improved condition	NaCl concentration (mM)
A, E	0
B, F	50
D, H	300

Illustration of an example for an iterative approach to the Buffer Exploration Kit, as outlined in the respective protocol. Note how all results from STEP2 and STEP3 would be compared to the best result from STEP1 (in this example well G1).



5. Safety information

In accordance with European Directive 1999/45/EC and Regulations 1907/2006/EC and 1272/2008/EC this product does not require a Material Safety Data Sheet as it contains a quantity not exceeding 1% of components classified as dangerous and not greater than 0.1% of components classified as carcinogenic. For this reason, NanoTemper Technologies provides no safety data sheet for this product. Nevertheless, when working with these or any other chemical it is always advisable to use protective gloves, suitable clothing and goggles. NanoTemper Technologies assumes no liability for damage to property and / or injury resulting from use and contact with this product.

6. Purchaser notification

NanoTemper grants the buyer the non-transferable right to use the purchased product for research conducted by the buyer. The buyer cannot sell or otherwise transfer this product or its components for commercial purposes.

Limited warranty.

NanoTemper will replace any product that does not meet the specifications. This warranty limits NanoTemper's liability only to the cost of the product when within the expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. NanoTemper assumes no responsibility or liability for any indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive.

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Contact

NanoTemper Technologies GmbH

Global headquarters:
Floessergasse 4
81369 Munich
Germany
Phone: +49 (0)89 4522895 0
Fax: +49 (0)89 4522895 60

info@nanotempertech.com
nanotempertech.com

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