Buffer and sample preparation for direct binding assay in 2% DMSO

GE Healthcare recommends either 10 mM or 20 mM phosphate buffer with 0.05% P20 for work with small molecule assays in Biacore systems. Detergent should be included unless there is a good reason to exclude it (e.g. detergent-sensitive ligands).

Use the stock solution 10 x PBS-P+ (with 0.5% P20) provided by GE Healthcare to prepare running buffers and samples according to the description below. This buffer is designed to yield pH 7.4 when diluted 10 times with Milli-Q water and supplemented with 2% DMSO. Addition of other DMSO concentrations will slightly alter pH of the diluted buffer. Figure 1 illustrates pH as a function of DMSO concentration.

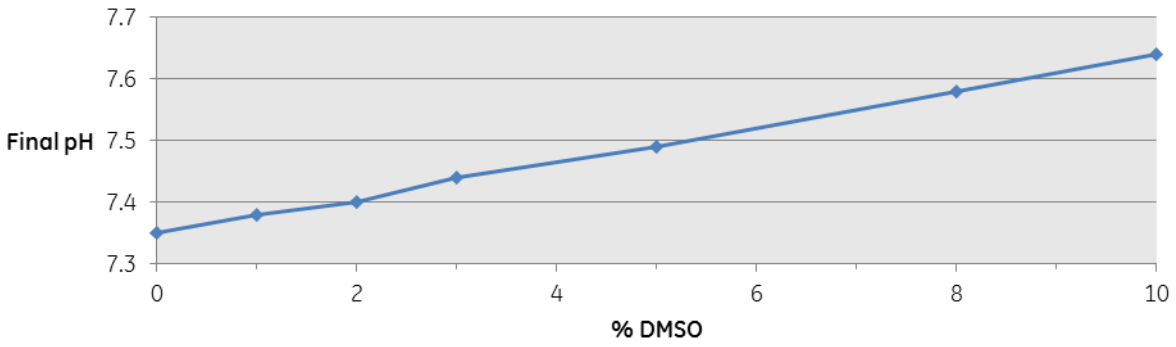

Figure 1. pH as a function of DMSO concentration. pH in a 10 x diluted PBS-P+ buffer supplemented with different amounts of DMSO.

Protocol

1. **Preparation of 2 litres 1.02 x PBS-P+:** Dilute 204 ml 10 x PBS-P+ stock to 2000 ml with Milli-Q water. This buffer will be used as running buffer during immobilization and for the preparation of solvent correction stock solutions, assay running buffer and samples.
2. **Preparation of solvent correction stock solutions and assay running buffer**: Prepare 10 ml of solvent correction stock solutions with 1.5% and 2.8% DMSO and 1 litre of assay running buffer with 2% DMSO, according to Table 1. Buffers and solutions need to be freshly prepared every day.

Table 1. Solutions for solvent correction and 2 % DMSO running buffer.

	1.5% DMSO	2.8% DMSO	2.0% DMSO running buffer
1.02 x PBS-P+	9.8 ml	9.8 ml	980 ml
100% DMSO	0.15 ml	0.28 ml	20 ml
Final volume	~10 ml	~10 ml	1000 ml

3. **Preparation of solvent correction working solutions:** Using the 1.5% and 2.8% DMSO stock solutions, prepare a series of aliquots for the solvent correction curve, according to Table 2 (volumes given in µl). Aliquots need to be freshly prepared every day.

Table 2. Preparation of solvent correction solutions. Volumes given in µl.

Buffer/Vial	1	2	3	4	5	6	7	8
1.5% DMSO	0	200	400	600	800	1000	1200	1400
2.8% DMSO	1400	1200	1000	800	600	400	200	0

The 8 solvent correction solutions should cover a range from approximately -500 RU to approximately +1000 RU relative to the baseline of the running buffer. To position the range of correction solutions and samples prior to assay start, use manual run (Biacore 2000, Biacore 3000, Biacore X100, Biacore T100 and Biacore T200) to inject the highest (2.8%) and lowest (1.5%) solution and a negative sample (prepared as the samples, do not use running buffer) over the surface. This will mimic the dilutions of the controls and real samples. Check that samples fall within the correction range.

4. **Sample preparation:** Prepare your samples so that the DMSO concentration will be 2%. Depending on the sample stock concentration, tendency to aggregate and size of library (number of samples) this procedure may differ.

Small to medium size compound libraries (few samples)

– For example, dilute the sample stock (in 100% DMSO) solution 50 times to obtain a DMSO concentration of 2%. For 1000 µl, mix 20 µl of sample stock with 980 µl of 1.02 x PBS-P+. If the sample stock is 10 mM, this dilution will result in a sample concentration of 200 µM. To prepare a concentration series dilute the sample further using assay running buffer (PBS-P+ with 2% DMSO). An example is shown in Figure 2.

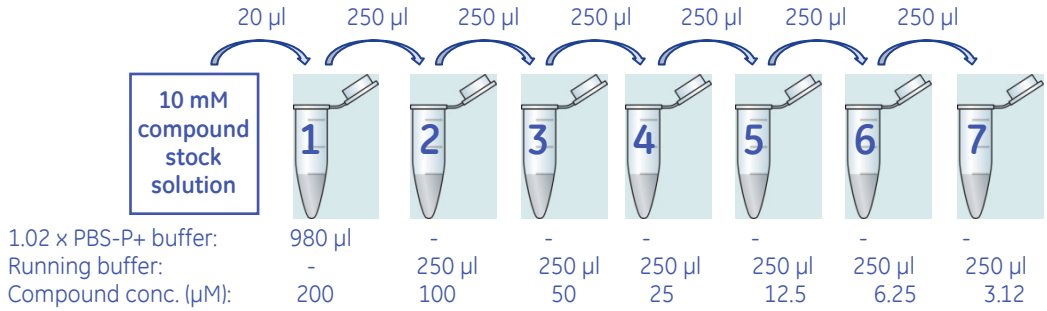

Figure 2. Dilution example for concentration series 3.12 – 50 µM. Concentrations 50 - 3.12 could be used for a kinetic analysis.

– Some samples may aggregate when diluted directly down to 2% DMSO; you may need to add an extra dilution step, e.g. dilute the sample stock with 100% DMSO to lower the sample concentration, then dilute further to obtain a DMSO concentration of 2% and a suitable sample concentration.

Large compound libraries (many samples)

– For example, dilute the sample stock (in 100% DMSO) solution 50 times to obtain a DMSO concentration of 2%. For 100 µl, mix 2 µl of sample stock with 98 µl of 1.02 x PBS-P+. If the sample stock is 10 mM, this dilution will result in a sample concentration of 200 µM. To prepare a concentration series dilute the sample further using assay running buffer (PBS-P+ with 2% DMSO).

– By using a specific dilution buffer you can speed up the sample preparation (since only one dilution step is required) and still maintain quality. It is important to use the dilution buffer for all samples including the negative controls. The composition of the dilution buffer is running buffer (0.5 x total volume) mixed with 1.02 x PBS-P+ (0.5 x total volume - sample volume), see example below.

Example: Dilute 384 samples (10 mM stocks in 100% DMSO) 100 times to 100 µM in a 384 well plate. Since the volume needed in this example is 100 µl per well (1 µl sample in 99 µl dilution buffer) approximately 50 ml dilution buffer is enough for all samples (0.1 ml x 384 =38.4 ml) and a number of negative controls. This gives a total sample volume of 0.5 ml in 49.5 ml dilution buffer.

1. Prepare the dilution buffer by mixing 25 ml running buffer (0.5 x 50 ml = 25 ml) with 24.5 ml 1.02 x PBS-P+ (0.5 x
2. 50 ml – 0.5 ml = 24.5 ml).
3. Mix 1 µl sample with 99 µl specific dilution buffer in each well. It is important to directly mix thoroughly in the well and not wait until all wells are prepared. Repeat for all samples.
4. Mix 1 µl 100% DMSO with 99 µl specific dilution buffer to prepare the negative control.
5. Centrifuge the 384 well plate for a short time (e.g. 1 min) to remove air.

Note: *It could be wise to practise the sample preparation in a 384-well plate prior to using the real samples, in order to find out how far down (how close to the bottom) the pipette tips should go when mixing.*

Important considerations

- Contaminations from glassware and plastic vials can affect the results. Glass bottles are recommended. Wash the glassware carefully with 50 mM NaOH followed by Milli-Q water before use. Avoid using dishwasher if possible.
- Make sure that all vessels and equipment are resistant to DMSO (use polypropylene plates). Use Teflon or nylon membranes to filter DMSO solutions (do NOT use cellulose acetate membranes).
- DMSO from different suppliers may vary in quality. Good results have been obtained at GE Healthcare using DMSO (analytical reagent grade, max 0.03% H_2O) from Sigma/Riedel de Haën (cat # 34943).
- It is important to use fresh DMSO from the same bottle when preparing solutions and samples within the assay.
- Always use the same 1.05 x PBS-P+ buffer to prepare solvent correction stock solutions, assay running buffer and samples.
- Always use filtered (0.22 M) and degassed immobilization and assay running buffer.
- Always run negative controls in all assays. Prepare the negative control in exactly the same way as the samples.

Note: *The running buffer can be used for start-up cycles but NOT as negative controls.*

Order code:

10 x PBS-P+ buffer	Order code: 28-9950-84

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