Procedure

Buffer and sample preparation for Biacore direct binding assay in 5% DMSO

We recommend either 10 mM or 20 mM phosphate buffer with 0.05% of 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 PBS-P+ 10× (with 0.5% P20) provided by Cytiva to prepare running buffers and samples according to the description below. This buffer yields approximately pH 7.49 when diluted 10 times with Milli-Q™ water and supplemented with 5% dimethyl sulfoxide (DMSO). Addition of other DMSO concentrations will slightly alter pH of the diluted buffer. Figure 1 illustrates pH as a function of DMSO concentration.

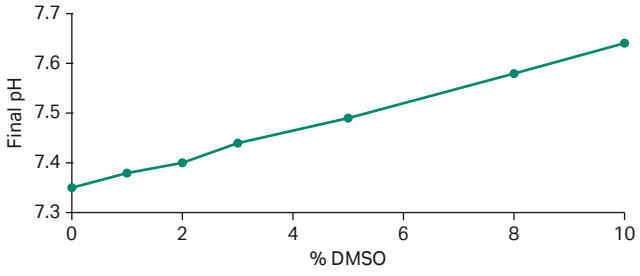


Fig 1. pH as a function of DMSO concentration. pH in a 10× diluted PBS-P+ buffer supplemented with different amounts of DMSO.

Protocol

1. **Preparation of 2 L of 1.05× PBS-P+:** Dilute 210 mL of 10× 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 4.5% and 5.8% DMSO and 1 L of assay running buffer with 5% DMSO, according to Table 1. Buffers and solutions need to be freshly prepared every day.

Table 1. Solutions for solvent correction and 5% DMSO running buffer

	4.5% DMSO	5.8% DMSO	5.0% DMSO running buffer
1.05× PBS-P+	9.5 mL	9.5 mL	950 mL
100% DMSO	0.45 mL	0.58 mL	50 mL
Final volume	~ 10 mL	~ 10 mL	1000

3. **Preparation of solvent correction working solutions:** Using the 4.5% and 5.8% 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

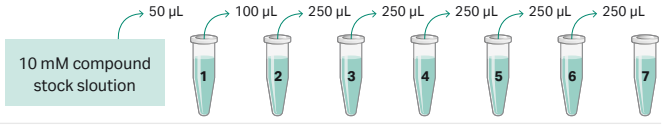
Buffer/Vial	1	2	3	4	5	6	7	8
4.5% DMSO	0	200	400	600	800	1000	1200	1400
5.8% DMSO	1400	1200	1000	800	600	400	200	0

The eight 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 X100, Biacore S200, and Biacore T200) to inject the highest (5.8%) and lowest (4.5%) 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 5%. 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 20 times to obtain a DMSO concentration of 5%. For 1000 µL, mix 50 µL of sample stock DMSO and 950 µL of 1.05× PBS-P+. If the sample stock is 10 mM, this dilution will result in a sample concentration of 500 µM. To prepare a concentration series dilute the sample further using assay running buffer (PBS-P+ with 5% DMSO). An example is shown in Figure 2.
- Some samples may aggregate when diluted directly down to 5% DMSO; you may need to add an extra dilution step, for example, dilute the sample stock with 100% DMSO to lower the sample concentration. Dilute further to obtain a DMSO concentration of 5% and a suitable sample concentration.



1.05× PBS-P+ buffer (µL)	950	–	–	–	–	–	–
Running buffer (µL)	–	400	250	250	250	250	250
Compound conc. (µM)	500	100	50	25	12.5	6.25	3.12

Fig 2. Dilution example for concentration series from 500 to 3.12 µM. Concentrations of 50–3.12 µM could be used for a kinetic analysis.

Large compound libraries (many samples)

- For example, dilute the sample stock (in 100% DMSO) solution 20 times to obtain a DMSO concentration of 5%. For 100 µL, mix 5 µL of sample stock with 95 µL of 1.05× PBS-P+. If the sample stock is 10 mM, this dilution will result in a sample concentration of 500 µM. To prepare a concentration series, dilute the sample further using assay running buffer (PBS-P+ with 5% 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 × total volume) mixed with 1.05× PBS-P+ (0.5 × total volume - sample volume), see example below.

Example: Dilute 384 samples (10 mM stocks in 100% DMSO) 20 times to 500 µM in a 384-well microplate. Since the volume needed in this example is 100 µL per well (5 µL of sample in 95 µL of dilution buffer) approximately 50 mL dilution buffer is enough for all samples (0.1 mL × 384 =38.4 mL) and a number of negative controls. This gives a total sample volume of 2.5 mL in 47.5 mL of dilution buffer.

1. Prepare the dilution buffer by mixing 25 mL running buffer (0.5 × 50 mL = 25 mL) with 22.5 mL of 1.05× PBS-P+ (0.5 × 50 mL – 2.5 mL = 22.5 mL).
2. Mix 5 µL of sample with 95 µL of 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.
3. Mix 5 µL of 100% DMSO with 95 µL of specific dilution buffer to prepare the negative control.
4. Centrifuge the 384-well microplate for a short time (e.g., 1 min) to remove air.

Note: *Test the sample preparation in a 384-well microplate prior to using the real samples 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 polytetrafluoroethylene (PTFE) 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 Cytiva using DMSO (analytical reagent grade, max. 0.02% H2O) from Honeywell/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× PBS-P+ buffer to prepare solvent correction stock solutions, assay running buffer and samples.
- Always use filtered (0.22 µm) and degassed immobilization and assay running buffer. Filtering and degassing are not necessary when using stock buffer solutions from Cytiva.
- If the pH of the assay running buffer (1.05×PBS-P+ with 5% DMSO) is suboptimal for the assay, adjust the pH to 7.4 using HCl.
- Always run negative controls in all assays. Prepare the negative control in the same way as the samples.

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

Ordering information

Product	Product code
PBS-P+ buffer 10×	28995084

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