Procedure

Preparation of 4-point solvent correction and samples for binding assay in 5% DMSO

Cytiva recommends either 10 or 20 mM phosphate buffer with 0.05% Surfactant 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., if the ligand is detergent-sensitive). Use the stock solution PBS-P+ 10× (with 0.5% P20) from Cytiva to prepare running buffers and samples according to the description below.

Protocol

1. **Preparation of 2 L of 1.05× PBS-P+:** Dilute 210 mL 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 6.0% 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

Nominal DMSO concentration	4.5% DMSO (~ 10 mL)	6.0% DMSO (~ 10 mL)	5.0% DMSO running buffer (1000 mL)
1.05× PBS-P+	9.5 mL	9.5 mL	950 mL
100% DMSO	0.45 mL	0.60 mL	50 mL

3. **Preparation of 4-point solvent correction working solutions:** Using the 4.5% and 6.0% DMSO stock solutions, prepare the aliquots for the solvent correction curve, according to Table 2. Aliquots need to be freshly prepared every day.

Table 2. Preparation of 4-point solvent correction solutions

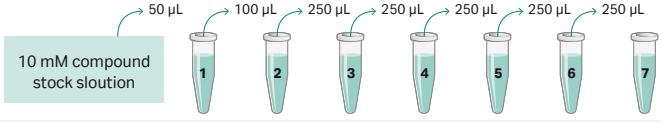
Buffer/Vial	1	2	3	4
Nominal DMSO concentration	6.0%	5.5%	5.0%	4.5%
4.5% DMSO	0	1500 µL	2 × 1500 µL	3 × 1500 µL
5.8% DMSO	3 × 1500 µL	2 × 1500 µL	1500 µL	0

The 4-point solvent correction solutions should cover a range from approximately -500 RU to approximately +1000 RU relative to the baseline of the running buffer.

4. **Sample preparation:** Prepare your samples so that the DMSO concentration will be 5%. Depending on the sample stock concentration, the 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-fold to obtain a DMSO concentration of 5%. For 1000 µL, mix 50 µL of sample stock with 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 1.



	1	2	3	4	5	6	7
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 1. Dilution example for concentration series of 500 to 3.12 µM. Concentrations in this concentration series could be used for a kinetic analysis.

- Some samples may aggregate when diluted directly down to 5% DMSO. An extra dilution step may be needed, for example, dilute the sample stock with 100% DMSO to lower the sample concentration, then dilute further to obtain a DMSO concentration of 5% and a suitable sample concentration.

Large compound libraries (many samples)

- For example, dilute the sample stock (in 100% DMSO) solution 20-fold 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).

Ordering information

Product	Product code
PBS-P+ buffer 10×	28995084

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