

## 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

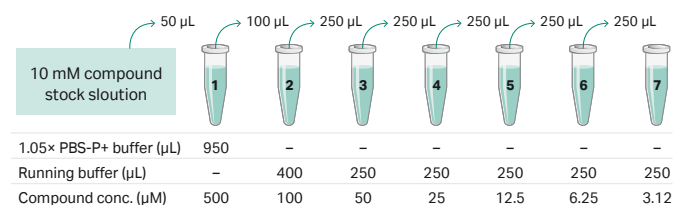
Buffer/Vial	1	2	3	4
Nominal DMSO concentration	6.0%	5.5%	5.0%	4.5%
4.5% DMSO	0	1500 µL	2 × 1500 µL	3 × 1500 µL
5.8% DMSO	3 × 1500 µL	2 × 1500 µL	1500 µL	0

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.



**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  $\mu$ L, mix 5  $\mu$ L of sample stock with 95  $\mu$ L of 1.05 $\times$  PBS-P+. If the sample stock is 10 mM, this dilution will result in a sample concentration of 500  $\mu$ M. 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 $\times$	28995084

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