

## GUIDELINES FOR BIOCHEMICAL SCREENING SERVICES

### 1. SCREENING PROJECT FORM (SPF)

Each biochemical kinase screening project with ProQinase will require a completed SPF providing important project details, such as i) number of compounds, ii) number and identity of kinases iii) concentration range, and iv) number of replicates.

Please send an electronic copy of the SPF via email before sending your compounds, and in addition enclose a signed copy with your compound shipment.

### 2. COMPOUND SUBMISSION

Compounds may be provided as solid or liquid samples. Using 100% of DMSO, ProQinase will – if needed – generate a 100x stock solution of

- (i) the final assay concentration for single concentration measurements
- or (ii) the highest concentration to be used in IC<sub>50</sub> determinations.

For IC<sub>50</sub> determinations, 100x stock solutions will be further diluted semi-log stepwise by ProQinase.

Please ensure that all labels used on vials and plates are inert to DMSO.

#### **a. Dry samples in vials**

Each compound should be provided in one vial (max. 80 vials, unambiguously labeled, preferably “1” to “x”) containing defined pre-weighed quantities (min. 0.5 mg) sufficient to achieve  $\geq 100\mu\text{L}$  of a 100x stock solution per singlicate testing against a set of up to 32 kinases<sup>1</sup>.

Ensure that vials are suitable for the addition of the calculated DMSO volumes. Please provide related information through the Compound Submission Form (CSF).

#### **b. Liquid samples in vials**

Each compound should be provided in one vial<sup>2</sup> (max. 500 vials, unambiguously labeled, preferably “1” to “x”) containing quantities of  $\geq 100\mu\text{L}$  per singlicate testing against a set of up to 32 kinases as a 100x stock solution in 100% of DMSO<sup>1</sup>.

Please provide related compound information through the Compound Submission Form (CSF).

#### **c. Liquid samples in plates**

Compounds and DMSO controls (10 $\mu\text{L}$ /well) should be provided as 100x stock solution (in 100% of DMSO) in high-quality, V-shaped, 96-well, HTS-compatible, flat microtiter plates<sup>3</sup>. For sealing your plates, we recommend the use of MTP adhesive foil<sup>4</sup>.

For each set of up to 4 kinases, one separate stock solution plate will be required. For plate preparation, please follow the layout instructions depicted in figure 1 and barcode-label your plates using one of the following codes: Code 39 (preferred), Code 128, Codabar, Interleave 2 of 5, UPC, or EAN.

Please provide information about compounds (and DMSO controls), plate ID(s) and well coordinates through an electronic list or table (Excel file).

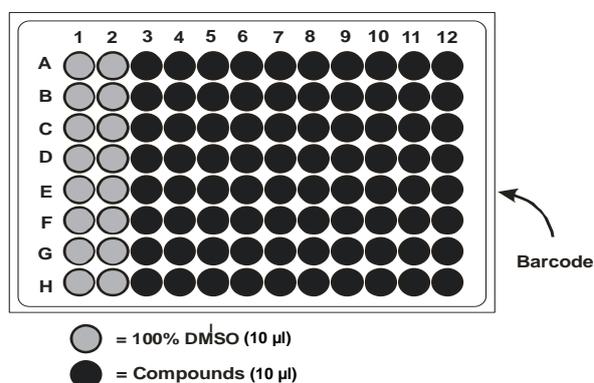
<sup>1</sup> Singlicate testing against 33–64 kinases requires  $\geq 200\mu\text{L}$ , against 65–96 kinases  $\geq 300\mu\text{L}$  etc.

<sup>2</sup> if two different concentrations of one compound should be tested, please provide two separate stock solutions)

<sup>3</sup> Polystyrene or polypropylene plates e.g. NUNC (#249570 or #442587) or GREINER (#65110 or #651201).

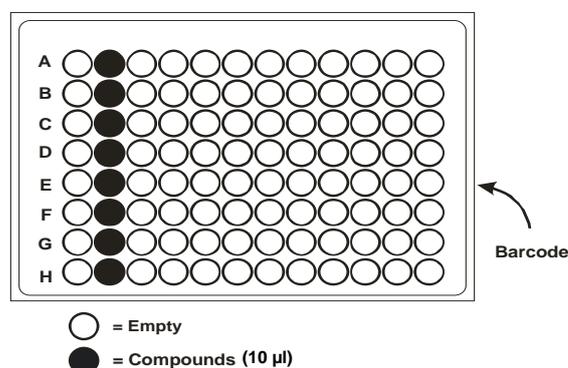
<sup>4</sup> e.g. Costar #6570, ABgene #0626 or Beckman #538619

A) Single concentration measurement



Provide up to 80 compounds per plate as 100x stock solutions (columns 3-12) and two control columns (100% of DMSO, columns 1 + 2)

B) IC<sub>50</sub> determination



Provide up to 8 compounds per plate (column 2) as 100x stock solutions of the highest assay concentration which will be diluted semi-log stepwise by ProQinase

**Figure 1:** Plate layout required for single concentration measurements and IC<sub>50</sub> determinations.

### 3. DUPLICATE TESTING

If the customer requests duplicate testing, ProQinase will per default perform duplicate testings in the same experiment. If you are interested in alternative types of duplicate testing, please inform ProQinase in advance as this might affect pricing and screening slot availability.

### 4. SHIPPING INSTRUCTIONS

For shipments, please enclose your vials or plates (stacked, covered with a lid<sup>5</sup> and wrapped) in a bag (cold resistant if sent on dry ice). Please include copies of the CSF/plate layout list and the signed SPF. For dry ice shipments, ensure to use thick wall styrofoam boxes filled with sufficient amounts of dry ice ( $\geq 5$ kg dry ice per prospective day of transportation).

To avoid unnecessary delay during shipment, we recommend to launch shipments on Mondays. All compounds of one project have to be sent in one shipment.

To simplify customs clearance and avoid customs duties, please state the **“TARIC Code 2942000000 (other organic compounds)”** within the customs documentation and indicate that the content is **“Only for laboratory testing, not for use in humans or animals, only smallest amounts and of no commercial value”**.

**Important:** Please use shipment terms DDP.

<sup>5</sup> e.g. NUNC #263339

Please ship compounds to:

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**GERMANY**

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## 5. COMPOUND ARRIVAL & HANDLING AT PROQINASE

ProQinase will inform you via email about the arrival of your compounds and the scheduled screening slot. Please ensure in advance that your compounds will tolerate the respective methods of compound handling (as outlined below) that will be performed at ProQinase (especially with respect to possible precipitation):

- a. Samples provided in vials will be
  - i. dissolved in 100% DMSO using the corresponding volume as stated in the CSF-document, to obtain 100x stock solutions (for dry samples only)
  - ii. additionally (for IC<sub>50</sub> determinations only) serially diluted through 10 semi-logarithmic steps using 100% DMSO (resulting in 10 different stock concentrations)
  - iii. further diluted 1:10 using H<sub>2</sub>O resulting in 10x compound stocks in 10% of DMSO  
(10 distinct stock solutions in case of IC<sub>50</sub> determinations)
  - iv. finally diluted 1:10 into the assay (resulting in the final assay concentration in 1% DMSO)
  
- b. Samples provided in plates will be
  - i. diluted 1:10 using H<sub>2</sub>O resulting in 10x compound stocks in 10% DMSO
  - ii. additionally (for IC<sub>50</sub> determinations only) serially diluted through 10 semi-logarithmic steps using 10% DMSO (resulting in 10 distinct concentrations in 10% DMSO)
  - iii. finally diluted 1:10 into the assay (resulting in the final assay concentration in 1% DMSO)