

## Guidelines: Compound Selectivity Profiling (IC<sub>50</sub> Profiling)

### Compound Preparation

1. Use microtiter plates that are suitable for automatic handling. For a detailed description of plate requirements refer to the SBS MTP Standards Dev. Committee Guidelines ([www.sbsonline.org](http://www.sbsonline.org)). In case of use of microplates not suitable for automatic handling loss of compounds due to robot failure may occur!
2. On the day of assay procedure at ProQinase compounds are handled as followed:
  - 100x compound stocks (in 100 % DMSO) will be diluted 1:10 with H<sub>2</sub>O (resulting in 10x compound stocks in 10 % DMSO)
  - 10x compound stocks (in 10 % DMSO) will be diluted serially (semi-logarithmic, 10 steps) with 10 % DMSO
  - 10x compound stocks (10 different concentrations, see above) are diluted 1:10 into the assay (resulting final assay concentration: 1x stock in 1 % DMSO)

Please make sure that your compounds stocks do not precipitate upon a 1:10 dilution with H<sub>2</sub>O!

3. Please provide compounds as one batch (i.e. no partial deliveries)

### Compound Selectivity Profiling

#### IC<sub>50</sub> Determination (10 concentrations, semi-log steps, n=1 or n=2)

(currently only performed in 96-well format)

- A. Use high-quality, V-shaped Polystyrene (e.g. Nunc #249570; GREINER #651101) or Polypropylene (e.g. Nunc # 442587; GREINER #651201) plates.
- B. Each plate can contain up to 8 compounds in column 2. Provide compounds as 100x stocks of the highest concentration to be tested, in 100 % DMSO.
- C. For measurements with n=2, provide compounds in separate wells in the following order:
 

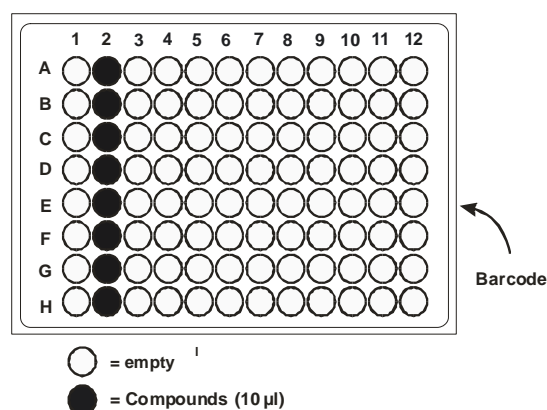
Compound 1:	A2B2
Compound 2:	C2D2
Compound 3:	E2F2
Compound 4:	G2H2
- D. Provide copies of each compound stock solution plate (10 µl compound stock solution each; 100x of concentration to be tested; in 100 % DMSO) as follows:

# of kinases	# of stock solution plates
1 – 8	1
9 – 16	2
17 – 24	3

etc.

- E. Attach barcodes on the right side of the plates; preferably Code 39, which is very fault-tolerant. Other

barcodes that can be used: Code 128, Codabar, Interleave 2 of 5, UPC and EAN.



- F. Please provide us with an electronic list of the compounds, plate ID(s) and well coordinates (for list format details, please see example on page 2).

## Guidelines: Compound Selectivity Profiling (IC50 Profiling)

### Compound Shipping Instructions

ProQinase's aim is to provide data of highest possible quality. Correct shipping and handling of test compounds is of extreme importance to reach this goal.

These shipping instructions are intended as a guideline and checking list for use by laboratory personnel shipping compounds to ProQinase. They are also available as a separate PDF file.

1. Use appropriate **high-quality, V-shaped, 96 well standard microtiter** plates for sample preparation! We recommend polystyrene or polypropylene plates e.g. NUNC (#249570 or #442587) or GREINER (#651101 or #651201).

Make sure that the plates (in particular polypropylene) are absolutely flat at room temperature after freezing on dry ice!

2. Use high quality MTP adhesive foil to seal the plates! Please make sure to use adhesive tape that does not detach on dry ice, e.g. Zymark #21640 or ABgene #0626 or Beckman #538619. Heat seals are also applicable, but be sure that they can be removed easily and plates are not deformed during the seal process!
3. Dry ice is very abrasive, in particular when partially evaporated during transport! To prevent the dry ice from detaching the adhesive tape, stack the plates, cover the topmost plate with a lid (e.g. NUNC #263339) and wrap the stack with rubber band. Put the stack in a bag made of cold-resistant material.
4. Include a completed and signed copy of the compound testing form in the dry ice package.

To ensure that nothing has been forgotten, the lower part of the electronic form is designed as a final check-list!

Please advise us of your shipment – preferably by faxing the completed compound testing form ahead of shipment!

Please additionally provide us with an electronic list of plate IDs and compound well coordinates (example given below):

MTP ID	Compound ID	Y-Pos	X-Pos
1234	XYZ12345671	A	2
1234	XYZ12345672	B	2
1234	XYZ12345673	C	2
1234	XYZ12345674	D	2
1234	XYZ12345675	E	2
1234	XYZ12345676	F	2
1234	XYZ12345677	G	2
1234	XYZ12345678	H	2

*etc.*

5. For shipment (in particular overseas), choose a carrier with demonstrated experience at the customs. Refill of dry ice in case of any unexpected delays must be ensured.

Preferably, start shipment at the beginning of a week to avoid delays over the weekend.

6. Ship compounds to:

**Dr. Frank Totzke**  
**HTS & Data Management**  
**ProQinase GmbH**  
**Breisacherstrasse 117**  
**D-79106 Freiburg**  
**GERMANY**

**Phone: ++49 (0)761 2061723**  
**FAX: ++49 (0)761 2061781 or 2061705**  
**mail: f.totzke@proqinase.com**

**Thank you very much for adhering to our guidelines!**