Puretitre

Caithness BIOTECHNOLOGIES

Natural compound library (PT200)

FOR RESEARCH USE ONLY

PRODUCT INFORMATION

Contents

- 3x 96-well microtitre plates, containing 200 pure natural compounds at 10 mM in dimethyl sulfoxide (DMSO).
- Product information sheet.
- Safety Data Sheet (SDS).

Intended Use

- For *in vitro* research purposes only.
- Warning: not for use in human or animal studies, this product must not come into contact with foods or drinks.
- Please read entire information sheet before using product.

HANDLING AND STORAGE

- Product is shipped on dry ice.
- Recommended storage temperature: -20°C or lower.
- Expiry date when stored at -20°C: 6 months from delivery.
- Expiry date when stored at -80°C: 18 months from delivery.
- Compounds may be light sensitive, protect from extended exposure to light.
- **DMSO and some of the compounds comprising the library can cause irritation to eyes and skin or by inhalation**. Avoid contact with eyes and skin, wear gloves and appropriate laboratory wear.

PRODUCT FORMAT

- Supplied as 100 µl per well in DMSO
- Bar codes (code 128) and human readable plate numbers are printed on all plates (plates are numbered sequentially PT01 to PT03).
- Plates are sealed with re-usable DMSO-resistant silicone cap mats.

ONLINE INFORMATION

Please visit www.caithnessbiotechnologies.com/data/ to download:

- List of all compounds included in the *Puretitre* library, with notes on the rationale for their inclusion in the library and reported biological properties (Adobe PDF format).
- Plate maps for locations of all compounds (MS Excel format).

BACKGROUND

Natural products have historically been the most successful source of new drugs, largely because compounds derived from natural sources occupy a chemical space with a far greater structural diversity than synthetic compound libraries, and tend to display superior ADME/T (absorption, distribution, metabolism, excretion and toxicity) properties. The *Puretitre* library aims to make natural product library screening accessible to smaller research groups in academia and industry, by optimising library size through a focus on natural compounds with established bioactivity and high structural diversity.

METHOD OF USE

The *Puretitre* natural product library may be used for many *in vitro* research purposes. However, the most commonly performed technique is likely to be screening for compounds which demonstrate biological activity against a particular target, receptor or phenotype, using a cell or protein-based assay in microplate format. The user should consider their requirements carefully before developing the assay, which should be optimised for their own purposes. The following suggestions are offered to aid the development of a simple screening protocol:

1) Safety precautions:

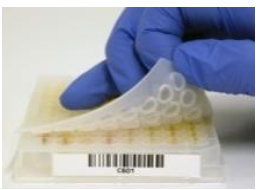
- DMSO, and some of the compounds comprising the library, can cause irritation to eyes and skin or by inhalation. Avoid contact with eyes and skin, wear gloves and appropriate laboratory wear.

2) Prior to screening:

- An assay should be developed in which the activity of the target of interest can be measured using a readout amenable to medium throughput screening (for example, this could be based on a transcriptionally-induced reporter enzyme, biochemical or fluorescence based measurements of enzyme activity, cellular proliferation or viability, ELISA of secreted proteins etc.).
- Prepare 3x 96-well microtitre plates containing the cells or reagents required to screen the *Puretitre* library (a volume of 100 - 200 µl per well is often appropriate).
- Every well in columns 2-11 of these plates should contain cells or reagents suitable for assay, and at least one additional column should be plated to allow for negative (DMSO only) and positive controls appropriate for the assay in question.

3) Defrosting the library:

- Allow *Puretitre* plates to defrost and then equilibrate to room temperature before removing sealing cap mats (NB DMSO remains solid below 19°C).
- Centrifuge library plates gently in pairs at 50 - 200 g for 1 minute, at the lowest setting of acceleration or deceleration if available, to settle the plate contents (otherwise droplets of solutions can remain on cap mats and significant volumes of solution can be lost).
- Note which corner of the plate locates the cap mat tab pull.
- Carefully remove silicone cap mats by pulling slowly from one corner, and store in a safe place with the plugs facing upwards for later re-use.



Tip: if solutions are observed on the upper surface of the plate after cap removal, wipe the plate surface with absorbent paper to avoid cross-contamination of wells.

(Continued overleaf).

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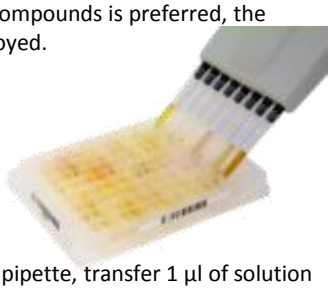
METHOD OF USE (continued from overleaf)

4) Challenging the target assay plate:

- If robotic transfer of library samples onto the target assay plate is preferred, please follow a protocol suitable for your equipment (plates are sequentially numbered from PT01 to PT03 in bar code 128).
- If manual transfer of library compounds is preferred, the following steps may be employed.
- Align the first of the library plates to be screened next to the first of the plates containing the target assay.
- Ensure both plates are in the same orientation (well A1 at top left).



- Using an 8-way multichannel pipette, transfer 1 µl of solution from each well of a single column (8 wells) from the library plate, and expel the aliquots into the medium of the corresponding column wells on the adjacent assay plate.

Tip: a common starting point in terms of dilution of compound onto the target assay is 1:100 (achieved by pipetting 1 µl of compound onto 99 µl target), but if too many hits are observed at this dilution, it may be useful to prepare daughter plates at 1:10 or further dilutions in DMSO for subsequent assays.

- Use fresh tips for each column to avoid cross-contamination between wells and back transfer of materials from the assay plate into the library plate.

Tip: to help keep track of which columns have been pipetted, a sterile plate lid can be used to cover previously used columns, or alternatively, pipetting past the first stop can introduce a bubble into the target plate wells which serves as a visible cue as to which wells have received treatments.

- Add 1 µl DMSO alone to several wells in columns 1 or 12 of the assay plate to serve as negative controls.
- Ideally, positive control agents, as appropriate for the assay in question, should also be pipetted into spare wells of the target assay plate.
- After the assay plate has been challenged with 80 compounds and controls, reseal the library plate using the same cap mat in the same orientation.
- Press firmly several times from the centre outwards to make a good seal.

Tip: DMSO is highly hygroscopic. To limit the absorption of moisture, which can accelerate degradation of bioactivity of compounds while in storage, ensure the plates are warmed to room temperature before opening, and replace cap mats immediately after aliquoting.

- Continue this process until all 3 library plates have been aliquoted onto target plates. Library plates can then be returned to storage at -20°C or -80°C.

METHOD OF USE

Identifying and taking hits forward:

- After a suitable period of incubation, measure the appropriate readouts for the target assay plate.
- Hits can be defined either on the basis of *a priori* performance goals (e.g. taking forward all those compounds which inhibit receptor activity by 80% or more), or percentage hit rate (e.g. taking forward the 20 compounds which display the highest level of inhibition).
- Remove from the stock library plates a small quantity of the positive compounds and aliquot these into a daughter plate of preliminary hits.
- Re-assay the preliminary hit compounds using a counter-screen assay to reveal those with non-specific activity.

Tip: the counter screen assay could be based, for example, on a related non-targeted receptor with the same readout, or the same target with a different readout method.

- Most researchers focus subsequent attention on those compounds which demonstrate high activity in the primary screen and low activity in the counter-screen.
- Resupply of any of the compounds present in the library is available from Caithness Biotechnologies Ltd or other suppliers

TECHNICAL HINTS AND LIMITATIONS

- Use kit by the expiry date (see 'Handling and Storage').
- If the primary screen yields too many hits, or background activity is excessive for many of the compounds, consider diluting the library 10x or further in DMSO and re-aliquoting in daughter plates before re-assay.
- Aliquoting the library into daughter plates may also be appropriate if many freeze-thaw cycles are expected.
- Avoid cross-contamination of wells by using tips once only.
- This product is for *in vitro* research purposes only, it must not be consumed or allowed to come into contact with foods or drinks.

WARRANTY

Subject to the due observance of the above installation, storage and maintenance instructions by the customer, the supplier will replace or refund the purchase price of any goods which the customer can establish are defective in workmanship, construction or materials. Claims for shortages, damage or defect must be made in writing within 10 business days of receipt of goods.

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