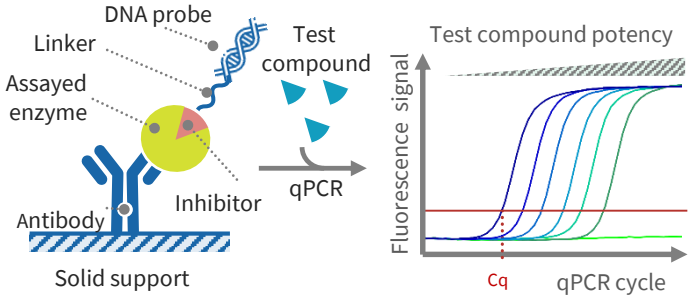
DIANA PNP ASSAY

for HTS screening and rapid profiling of PNP inhibitors

Purine Nucleoside Phosphorylase (PNP)

- PNP enzyme plays a crucial role in the **nucleotide recycling pathway** (converts inosine to hypoxanthin and guanosine to guanine)[1]
- PNP is overexpressed in lymphatic tissues, deficiency leads to **decline in T-lymphocyte** population, whilst B-lymphocytes remain unaffected[2]
- Inhibition of PNP is thus **attractive target against proliferation of T-cell malignancies**
- PNP inhibitor forodesine[3] already approved for treatment of peripheral T-cell lymphoma in Japan, yet suffering of **poor bioavailability**

DIANA Technology



DIANA (The **DNA**-linked **I**nhibitor **AN**tibody **A**ssay) is a novel technology for **ultrasensitive enzyme quantification** and for **high throughput screening** of enzyme inhibitors[4]. The inhibition potency of test compound is quantified via its ability to outcompete the DNA probe from the enzyme active site using qPCR readout.

3 potential applications

I. High throughput screening (HTS)

Efficient and sensitive sreening of libraries of compounds to identify novel inhibitors of PNP

II. Inhibitor profiling

Measuring PNP inhibition potency of compounds of interest, assisting in hit-to-lead development

III. ADME pharmacology testing

Determining set of *in vitro* pharmacological properties of PNP inhibitors (solubility, chemical and metabolic stability, membrane permeability,…)

Available in two formats

A. On-site service

- Run at DIANA Biotechnologies specialized facility
- Full data analysis and interpretation included
- Short turn-around times and competitive pricing
- In house diverse library of ~140k compounds available for HTS screening projects

B. Ready-to-use kit *(in development)*

- Profiling compounds for PNP inhibition
- Easy to implement in standard laboratory
- Assay plates, enzymes, probe, buffers, inhibitor standards & detailed protocol included

Key features of DIANA PNP assay

Economical	Minimal sample consumption (<0.05µL of 10 mM solution needed for K_i determination)
Quantitative	Accurate determination of K_i values from a single well measurement
Sensitive	Low pM to high µM K_i determination
Universal	The same assay can be used for HTS, affinity profiling and ADME testing
Robust	Z` factor ~ 0.95 and assay window > 10^4 - 10^5
Fast	Assay time < 3hrs
High Throughput	>100,000 cpds per day[£]

[£]assuming pooling of 10 cpds within one well

TA ČR

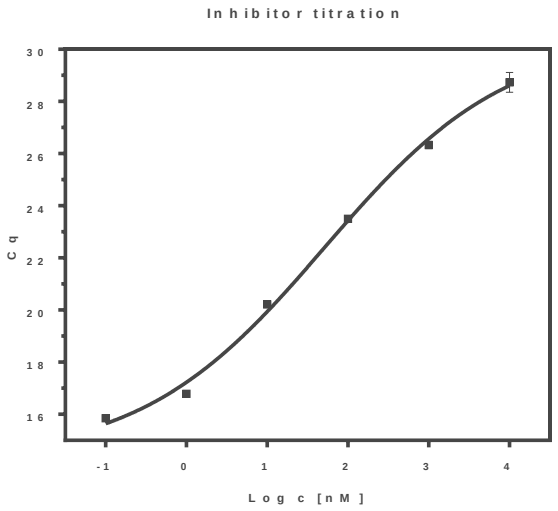
Development of the DIANA PNP assay has been co-financed with the state support from the Technology Agency of the Czech Republic, Program Zeta.

DIANA BIOTECHNOLOGIES

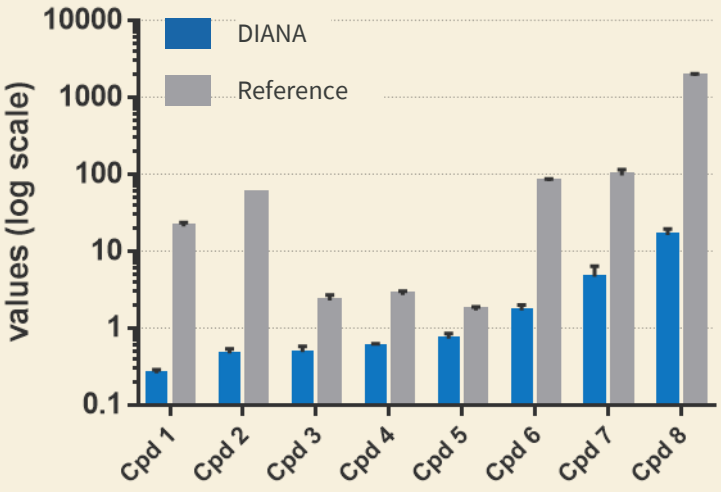
Dose response and calculation of Ki

Titration of PNP inhibitor to demonstrate the dose-response curve of the assay. Y-axes shows DIANA assay readout, i.e. Cq value, which is proportional to the enzyme inhibition. Note: DIANA read-out signal can also be transformed into classical vi/vo inhibition signal.

Due to the quantitative nature of DIANA assay, the Ki value of tested inhibitor can be precisely calculated from each single data point of the dose-response curve within the assay linear range (here 1 nM to 10,000 nM inhibitor).



Assay validation



To validate the assay accuracy, Ki measured by DIANA (calculated from Cq values determined at multiple inhibitor concentrations ranging from 0.1 to 10000 nM) were compared with reference IC50 values determined for the same compounds by radiometric method using radiolabeled substrate and product separation by TLC[5] (unpublished data from the Institute of Organic Chemistry and Biochemistry in Prague).

This data demonstrate that DIANA method is more sensitive compared to radiometric assay, while providing consistent readout across all the tested compounds.

FAQ

How DIANA calculates Ki values from single data point of inhibition curve?

In DIANA the free enzyme (not occupied by test compound) is measured directly, which corresponds to the vi value. Thanks to the logarithmic nature of the qPCR readout, the dynamic range of the assay is commonly several orders of magnitude. Such large dynamic range enables to calculate the Ki value from single point and make fitting of inhibition curves redundant.

What inhibitor concentration should be used for measurement to determine Ki correctly?

The concentration range depends on the Ki values of test compound. Generally, the lowest point of concentration range is close to the compound's Ki values and the highest concentration is approximately 100 – 1000 times higher (depending on the individual assay performance)

How many data points needs to be measured to reliably determine Ki values of test compound?

One data point is sufficient to obtain precise Ki value for test compound as long as the test concentration is within the assay range.

DIANA BIOTECHNOLOGIES

www.dianabiotech.com
info@dianabiotech.com
+420 212 247 340

References

1. Kim, B. K., Cha, S. & Parks, R. E. Purine Nucleoside Phosphorylase from Human Erythrocytes: I. PURIFICATION AND PROPERTIES. J. Biol. Chem. 243, 1763–1770 (1968).
2. Giblett, E., Sandman, R., Wara, D., Diamond, L. & Ammann, A. Nucleoside-phosphorylase deficiency in a child with severely defective T-cell immunity and normal B-cell immunity. The Lancet 305, 1010–1013 (1975).
3. Robak, P. & Robak, T. Older and new purine nucleoside analogs for patients with acute leukemias. Cancer Treat. Rev. 39, 851–861 (2013).
4. Navrátil, V., Schimer, J., Tykvart, J., Knedlík, T., Vik, V., Majer, P., Konvalinka, J., Šácha, P. DNA-linked Inhibitor Antibody Assay (DIANA) for sensitive and selective enzyme detection and inhibitor screening. (2017) Nucleic Acids Res.
5. Wortmann, R. L. et al. Purine Nucleoside Phosphorylase Deficiency. Arthritis Rheum. 22, 524–531 (1979).