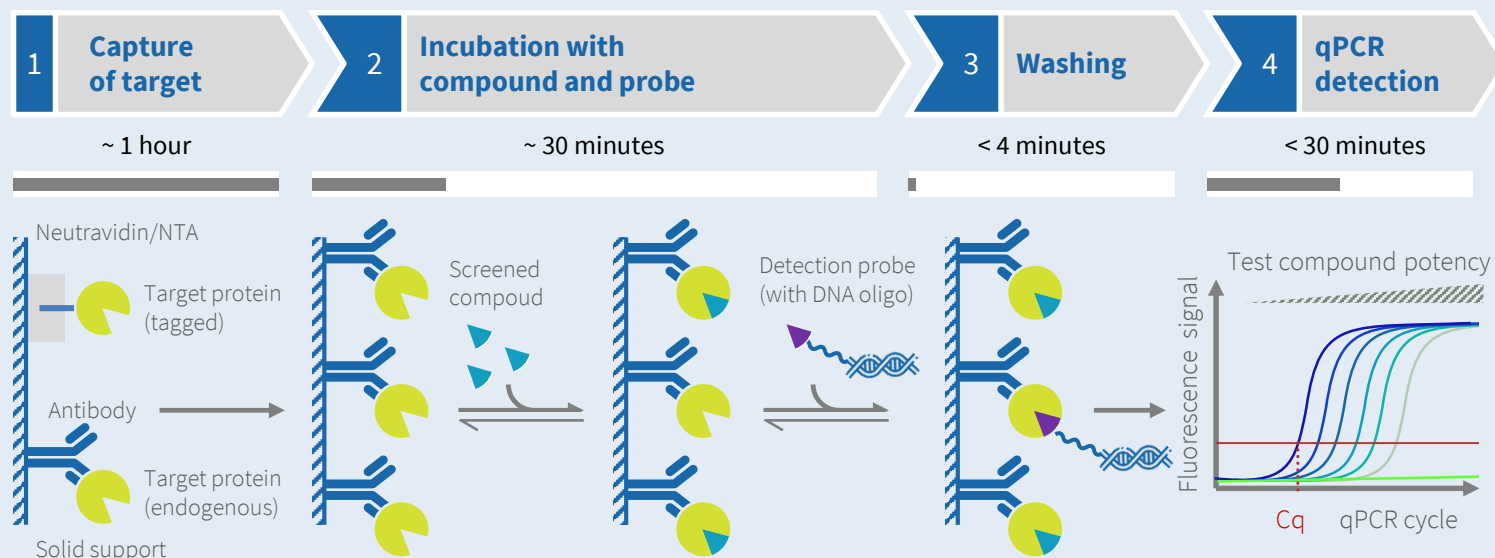


DIANA for High Throughput Screening

DIANA (The DNA-linked Inhibitor ANTibody Assay) is a novel multi-well plate format assay suitable for high throughput screening of enzyme inhibitors or ligands of other proteins (e.g. receptors or transporters).

DIANA protocol for High Throughput Screening



The target is captured to a solid phase via appropriate anchor and incubated with screened compounds together with the DIANA probe made of small-molecule inhibitor attached to a reporter DNA. The inhibition potency is directly determined using quantitative PCR. DNA-linked substrates or ligands can also be used as probes.

ADVANTAGES of DIANA for High Throughput Screening

- Suitable for screening of enzyme inhibitors, receptor ligands and protein-protein interaction inhibitors (PPIs)
- Available for panels of enzymes (i.e. Carbonic Anhydrases or methyltransferases) for subsequent selectivity profiling
- **Versatile:** Recombinant or purified target not necessary, micrograms of target may be sufficient for HTS
- **Robust:** signal-to-noise ratio of several logs ($Z' > 0.9$; $CV < 5\%$)
- **Quantitative Output:** inhibition potency accurately determined from a single-well measurement
- **Sensitive & Selective:** ultralow false positive and negative rates
- Pooling of compounds to boost throughput possible

Feature	Value
Assay window	Up to 6-logs
Assay format	384-well qPCR plates
Reagent volume	$\leq 5 \mu\text{l}$
Pooling of compounds	5-20/well
Throughput (per day)	>100,000

DIANA can be fully automated in any screening facility

- Robust simple protocol with few steps (similar to ELISA)
- Common liquid handlers and qPCR cyclers are suitable
- **BlueWasher from BlueCatBio** enables smooth automation: delivers fast and robust washing of 384-well qPCR plates with minimal residual liquid and minimal wash buffer consumption
- **Throughput:** 60 plates per 24 hours on one qPCR cycler
 - ~21,000 compounds/day/qPCR cycler
 - >400,000 compounds/day/qPCR cycler with pooled library



TWO OPTIONS for delivering the DIANA-based HTS projects

1 HTS assay kit

- Screening **performed at a customer site**
 - Easy to implement at any typical HTS screening facility or at smaller scale in academic labs
- **Kit components** delivered by DIANA Biotechnologies:
 - **Assay plates** (surfaces binding his-tag, GST-tag, biotin or precoated with selective antibody for unpurified targets)
 - **Enzyme/receptor/protein target**
 - **DIANA probe** designed for the target
 - Inhibitor standards for validation
 - Buffers, qPCR reagents
 - Detailed protocol
- Customer uses own compound library and performs data analysis

2 HTS screening service

- HTS project run at specialized **DIANA Biotechnologies screening facility**
- **In-house ~ 150,000 compound library** available for screening
 - Customer-provided or other third party compound libraries can also be used (pooled setup preferred for easier logistics and increased confidentiality)
- **Inhibition potency accurately determined** for each compound, hits ranked by affinity
- Assay validated with known inhibitors
- **Full data analysis** and interpretation included
- **Short turn-around times and competitive pricing**

Other related services

Custom assay development

- Lead time ~3 months, high success rates even for difficult targets
- Minimal performance criteria and risk-sharing models possible

DIANA-based ADME (*in development*)

- Typically performed on leads as a follow-up of the HTS project
- Synergies of using the same DIANA-based assay as for the HTS screening

Lead compound optimization

- Expertise in organic synthesis and medical chemistry

GROWING CATALOGUE OF TARGETS: Enzymes and Receptors

Application panels

Targets

Oncology	Glutamate carboxypeptidase II (GCPII, PSMA), Fibroblast activating protein (FAP), Carbonic Anhydrases IX and XII (CAIX and CAXII)
Neurology	Glutamate carboxypeptidase II (GCPII, PSMA) and III (GCPIII)
Energy metabolism	Fibroblast activating protein (FAP), Insulin receptor (INS-R)
Steroid Metabolism	Hydroxysteroid dehydrogenase 17 beta 1 (HSD17b1), steroid receptors (<i>in development</i>)
Influenza targets	Neuraminidase N1, PA-PB1 polymerase interaction, PB2 cap binding protein
Carbonic anhydrases	Selectivity profiling on panel of human Carbonic anhydrases
Kinases	Kinase panel for selectivity profiling (<i>in development</i>)
Methyltransferases	S-Ado-Met transferases (<i>in development</i>)
On demand targets	Assay can be developed on demand to majority of relevant protein targets

DIANA Biotechnologies s.r.o.

Nad Safinou II 366
252 50 Vestec
Czech Republic

www.dianabiotech.com
info@dianabiotech.com
+420 212 247 340
+420 608 371 211

