

# DIANA Carbonic Anhydrase Panel

DIANA (The DNA-linked Inhibitor ANtibody Assay) is a novel proprietary multi-well plate format assay suitable for selectivity profiling and **high throughput screening** of enzyme inhibitors.

## Human Carbonic Anhydrases are Medicinal Targets

- 12 active isoforms with diverse functions in human, cytosolic (CAI, CAII, CAIII, CAVII and CAXIII), membrane bound (CAIV, CAIX, CAXII and CAXIV), mitochondrial (CAVA and CAVB) and secreted forms (CAVI).
- All known potent Carbonic anhydrases (CAs) inhibitors are sulphonamide derivatives that inhibit most of the human isoforms (all CAs except CAIII are broadly inhibited by sulphonamides)
- Several drugs inhibiting Carbonic anhydrases (CAs): diuretics, anti-glaucoma, anti-epileptics or anti-convulsants
- Drugs inhibiting CAs suffer from various side effects due to inhibition of several CA isoforms and poor pharmacology
- No isoform specific inhibitors known
- CA-IX and CA-XII are promising anti cancer targets:
  - Essential for pH homeostasis of the cancer cells
  - SMDCs being developed, selective inhibitors needed

Desired effect	Targets
Diuretics (anti-oedema)	CAII and others
Anti-glaucoma	CAII (topical)
Anti-cancer	CAIX, CAXII
Side Effects	Off-targets
Depression, fatigue	Multiple
Weight loss (undesired); Anti-obesity (desired)	CAVA, CAVB and others

- Search for selective CA inhibitors has so far been hindered by the lack of suitable HTS assay and selectivity panels

## DIANA for HTS and Selectivity Screens of Anhydrase Inhibitors

- DIANA is the first assay suitable for both HTS and selectivity assessment of inhibitors among Carbonic Anhydrases
- HTS for any of the 11 sulphonamide susceptible isoforms (automatable 384-well)
- Subsequent selectivity profiling against all 11 isoforms (manual 96- or automatable 384-well)
- **Quantitative Output:** inhibition potency accurately determined from a single-well measurement
- **Robust:** signal-to-noise ratio of several logs ( $Z' > 0.9$ ;  $CV < 5\%$ )
- **Sensitive & Selective:** ultralow false positive and negative rates
- Pooling of compounds to boost throughput possible

Number of isoforms	11
Assay format	96- or 384-well
Reagent volume	5 or 20 $\mu$ l
Pooling of compounds	10-20/well
Throughput (per day)	>100,000

## DIANA CA selectivity panel – easy-to-use manual or automated kit

### Kit components delivered by DIANA Biotechnologies:

- Assay plates (precoated to bind anhydrases, 96- or 384-well)
- Selected (up to 11) isoforms of Human Carbonic Anhydrases
- DIANA probe mix, Inhibitor standards
- Buffers, qPCR reagents
- No special instruments needed for manual 96-well protocol:
  - qPCR cycler sufficient, whole assay completed in 2 hours
  - One assay plate = inhibition profile of 3 inhibitors

1. Enzyme/receptor capture	~1 hour
2. Incubate with DIANA probe and test compounds	~30 minutes
3. Wash	< 4 minutes
4. qPCR detection	< 30 minutes

## DIANA Carbonic Anhydrase Panel can be highly automated

- **Simple robust protocol**, similar to ELISA: (1) capture of the enzyme, (2) incubation with compounds and probe, (3) wash, (4) qPCR detection.
- **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 in wells and minimal wash buffer consumption (little waste)
- **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



## Carbonic Anhydrase affinity profiling also provided as a service

### CA affinity profiling service

- Run at specialized DIANA Biotechnologies screening facility
- Customer-provided compounds analyzed for affinity against **full panel of Human Carbonic anhydrases**
- **Inhibition potency of each CA accurately measured** and provided in easy-to-interpret format
- Assay validated with known inhibitors
- **Full data analysis** and interpretation included
- **Short turn-around times** and **competitive pricing**

### Other related services

- **Custom assay development** for new targets
  - Lead time ~3 months, high success rates
- **High throughput screening** for novel inhibitors
  - Using either **In-house ~ 150,000 compound library** or customer-provided libraries (pooled setup preferred for easier logistics and increased confidentiality)
- **DIANA-based ADME-tox testing** of lead compounds
- **Medicinal chemistry follow-up**

## 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> )
<b>On demand targets</b>	<b>Assay can be developed on demand to majority of relevant protein targets</b>

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