SIGNAXENO™

Human CYP450 essential

SIGNATOPE™

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SIGNATOPE GmbH
Markwiesenstr. 55 •
D-72770 Reutlingen
P + 49 7121 744 08 - 61

info@signatope.com
www.signatope.com

General management:
Dr. Oliver Poetz
Dr. Hannes Planatscher

Product specialist:
Dr. Helen Hammer
hammer@signatope.com
P + 49 7121 74408 - 63

WHY CYP PROTEIN ANALYSES?

- Protein levels reflect enzyme activity better than mRNA.
- Protein isoforms are unambiguously identified and quantified using mass spectrometry, CYP probes often lack isoform specificity.

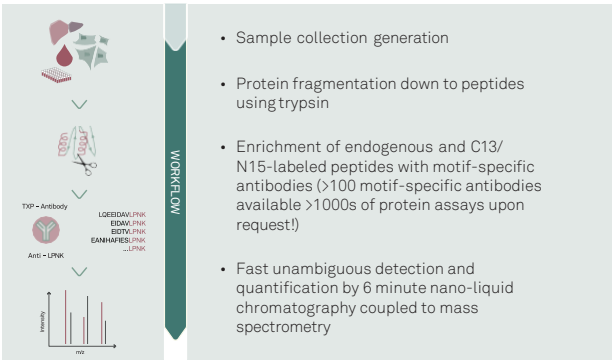
WHY SIGNATOPE?

- Cytochrome P450 isoforms are unambiguously identified and quantified by mass spectrometry; other methods lack of isoform selectivity.
- Workflow requires a minute amount of sample (96 well cell culture plate). Workflow allows analyzing hundreds of samples within a week, compared to standard targeted proteomics approaches.
- Batch processing concept includes calibration curves for CYP450 isoforms and biological quality control samples, making results absolute, reliable and reproducible.
- Validated according to FDA-guidance for bioanalytical method validation.
- Report as supportive data ready for submission.

INTRODUCTION

CYP enzymes are vital for drug metabolism, metabolizing various drugs and xenobiotics. Some drugs can affect CYP450 enzymes, potentially causing drug interactions. FDA guidelines mandate in vitro drug interaction studies during drug development to assess investigational drug induction potential for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, & CYP3A4, primarily evaluating mRNA levels. However, mRNA quantification may not accurately reflect enzyme function, leading to false-positive findings and unnecessary human DDI studies.[1,2] Our multiplex assays provide precise, isoform-specific protein levels for CYP enzymes, correlating with enzyme activity, ensuring reliable results.[3-6]

Complete your induction analysis with accurate protein data of the validated SIGNAXENO panels!



TECHNOLOGY

SIGNATOPE has a proprietary technology that combines motif-specific antibodies to capture CYP enzymes with mass spectrometry readout. These antibodies are designed to recognize and bind to the target CYP enzymes with high affinity, ensuring accurate quantification. The technology also provides high sensitivity, enabling for the detection of low abundance CYP enzymes in complex biological samples – tissues, cell pellets or lysates.[3,4,6,7] The final readout by mass spectrometry ensures definitive identification and quantification.

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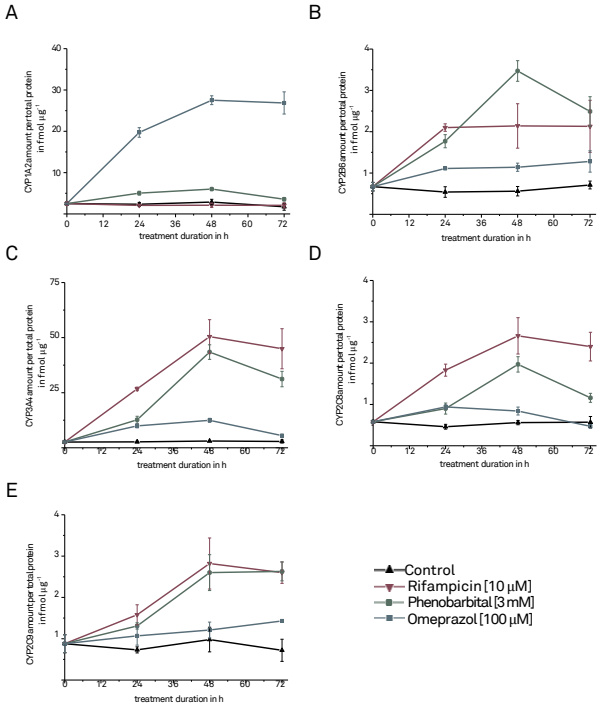
VALIDATED & RELIABLE RESULTS

We have extensively validated our assays to ensure reliable and reproducible results. The technology has been tested in various sample types, including tissues and cells, to ensure its applicability across different experimental setups.

		CYP1A2	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP3A4
Parameter	Acceptance criteria	Max (%)	Max (%)	Max (%)	Max (%)	Max (%)	Max (%)
Intra assay precision (% CV)	≤ 20%, ≤ 25% at LLOQ/ULOQ	10	11	10	6	9	5
Intra assay accuracy (% accuracy)	≤ ± 20%	5	11	14	2	5	6
Intra assay total error (% TE)	≤40%	15	17	25	9	12	10
Inter assay precision (% CV)	≤ 20%, ≤ 25% at LLOQ/ULOQ	19	18	20	19	17	19
Inter assay accuracy (% accuracy)	≤ ± 20%	4	6	6	5	3	2
Inter assay total error (% TE)	< 40%	23	23	24	21	20	21

INDUCTION STUDIES

Human cryopreserved hepatocytes were cultivated in 96-well plates. Cells were seeded at the same time and treated with DMSO (control), rifampicin (10 µM), phenobarbital (3 mM), and omeprazole for 72h, 48h, 24h and 0h. The CYP enzymes (A)1A2, (B)2B6, (C)2C8, (D)2C9, and (E)3A4 were quantified (biological replicates, n=3). The protein expression after cultivating cells for 72h using maintenance medium, is shown as base level (0h). CYP2C19 protein was not expressed in this donor.



EXPERT SUPPORT & GUIDANCE

We provide expert support and guidance throughout the quantification process. Our team of scientists can assist you in experimental design, sample preparation, and data analysis, ensuring that you obtain meaningful and interpretable results. This support can be particularly valuable if you are new to CYP enzyme quantification or require assistance in optimizing your experimental workflow.

SAMPLE REQUIREMENTS

Sample type	Amount	Storage temperature	Shipping temperature
Cell pellet	10 µg protein, app. 10.000 cells	-80 °C	on dry ice
Cells on membrane	10 µg protein, app. 10.000 cells	-80 °C	on dry ice
Cell lysate	10 µg protein extract, at 0.5 µg/µL	-80 °C	on dry ice

LITERATURE

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4. Wegler, C. et al. Variability in Mass Spectrometry-based Quantification of Clinically Relevant Drug Transporters and Drug Metabolizing Enzymes. Mol Pharm 14, 3142-3151 (2017).
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7. Iversen, D.B. et al. Flucloxacillin Is a Weak Inducer of CYP3A4 in Healthy Adults and 3D Spheroid of Primary Human Hepatocytes. Clin Pharmacol Ther (2023).