

CYP450 Protein Assay – Human Induction Kit Extended Panel

Quantitation of Cytochrome P450 Isoforms using a SCIEX Triple Quad™ or QTRAP® System

The Cytochrome P450 (CYP) proteins are a family of membrane bound proteins that function as a major metabolizing enzyme in the human body. Quantification of CYP induction is critical in determining the disposition, safety and efficacy of drugs. It is advantageous to measure actual protein levels when performing such studies, however traditional Western blot analysis has drawbacks due to isoform specificity and speed of analysis. A gel-free high-throughput LC-MS approach to quantitate the CYP isoforms 1A2, 2B6, 3A4 and 3A5 was developed¹ and commercialized in 2011 (CYP450 Human Induction kit, 4445252, www.sciex.com). Expanding on this initial offering, a larger panel has been developed which allows the simultaneous quantification of seven CYP isoforms, 1A2, 2B6, 2C9, 2C19, 2E1, 3A4 and 3A5.

Key Features of the CYP450 Protein Assay – Human Induction Extended Panel

- Enables measurement of CYP 1A2, 2B6, 2C9, 2C19, 2E1, 3A4 and 3A5
- Stable isotope labeled peptides are supplied as internal standards for quantification of each protein isoform.
- The assay can be performed using microsomal or S9 fractions from human hepatocytes or cell lines
- Works with current LC-MS/MS instrumentation already used for enzyme activity assays and inhibition studies
- Multiple Reaction Monitoring combined with liquid chromatography separation (LC-MRM) is used in this protein assay to provide very specific detection and quantification of isoform-specific peptides in a complex cell matrix.

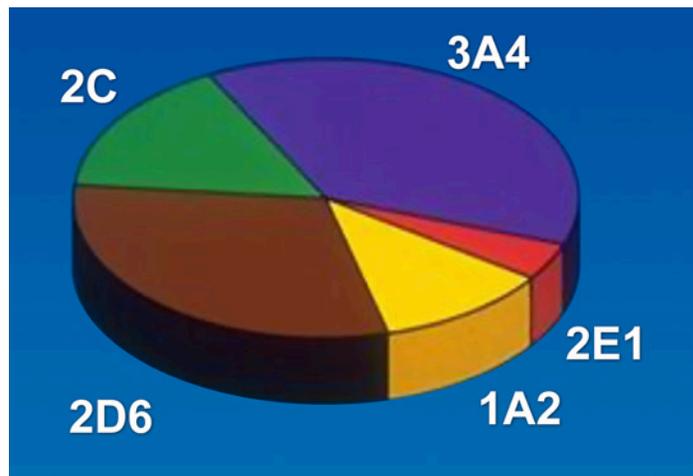


Figure 1. 3A4, 2D6, 1A2 and 2Cs are the major isozymes that metabolise the drugs on the market. 2B6 is also important because it is inducible and drug-drug interaction potential. 2D6 is thought not to be inducible and therefore not typically studied.

Kit Contents

Starter Kit Content

- Stable Isotope labeled peptide standards for seven CYP450 isoforms: 1A2, 2B6, 2C9, 2C19, 2E1, 3A4 and 3A5 (3 peptides for each isoform)
- Peptide C18 column (2.1mm x 5 cm)
- All sample preparation buffers and reagents (for denaturation, reduction, alkylation, trypsin digestion and dilution)
- Acquisition methods
- Data processing and report templates

User Supplied Content

- MultiQuant Software 1.2 or higher
- Microsoft Excel Software 2007 or higher
- SCIEX Triple Quad or QTRAP system
- Analyst® Software (version 1.5 or higher preferred)
- LC System (kit designed to be used with high flow chromatography, but method can be adapted for lower flow)

Protocol

The kit is designed to work with standard workflows typically employed for induction studies. For example, following enzyme activity assays or mRNA assays from a 96 well or 24 well plate, the same cells can be prepared for LC-MRM protein analysis (Figure 2).

- S9 or microsomal subcellular fractions are prepared by lysing the cells and performing one or more steps of centrifugation.
- For best results 1 x 10⁶ cells per analysis are recommended (100 ug total protein). Sample may be pooled.
- Time of sample preparation is 2 hours plus 4 hrs for tryptic digestion
- Samples are analysed using the SCIEX Triple Quad™ or QTRAP® Systems.
- Time of LC-MS analysis is 15 minutes per sample using conventional HPLC analysis.
- Data is processed with MultiQuant™ Software and Microsoft Excel.
- Protein expression changes automatically computed and graphically displayed.

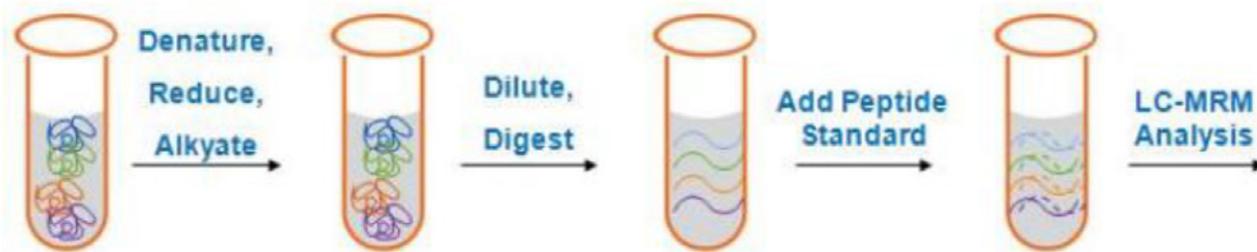


Figure 2. Simple Sample Preparation Protocol. Protein fraction is denatured, then disulfide bridges are reduced and alkylated. Solution is diluted to reduce the denaturant concentration before protein digestion. Trypsin is added and the proteins are digested for a few hours. The stable isotope labeled peptide internal standards (heavy peptide solution) are added and sample is submitted for LC-MRM analysis.

CYP450 Peptide Standards

The kit contains three peptide standards for each protein, synthesized using isotopically enriched amino acids, lysine and arginine. Peptides and MRM transitions were chosen based upon several criteria:

- Uniqueness of peptide sequence to a specific isoform
- Consistent and reproducible digestion behavior with no multiple K and R in series (which can digest incompletely)
- Minimum number of residues that are prone to chemical and post translational modifications
- Good MS ionization and gas phase fragmentation
- Chromatographic reproducibility
- No interferences in MRM transitions

An example of a typical LC-MRM analysis of the 21 isotopically enriched peptides to quantitate the CYP isoforms 1A2, 2B6, 2C9, 2C19, 2E1, 3A4 and 3A5 is shown in Figure 3.

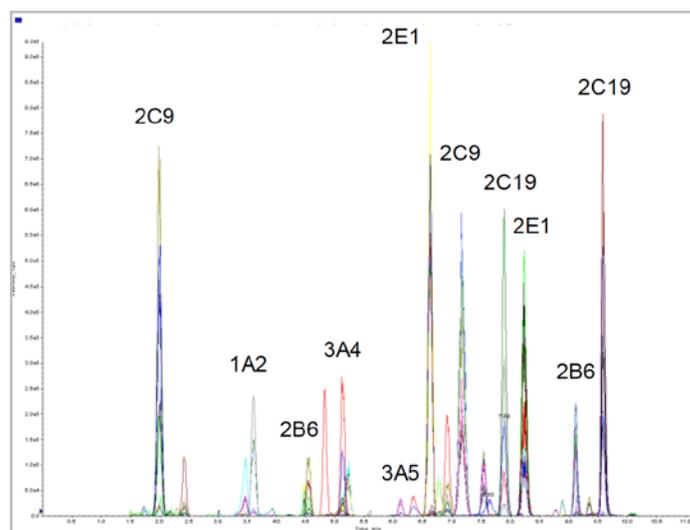


Figure 3. Typical LC-MRM Analysis of the CYP450 expanded panel peptide standards. The majority of the peaks are labeled with the CYP isoform they represent

Software for Data Processing

Once the acquisition is complete, data is processed with MultiQuant™ Software. The software automatically integrates all the MRM data and computes area ratios to the added peptide internal standard for all samples. Processed area ratios results are exported and imported into a Microsoft Excel template for computation of protein level data. Results can be plotted in a simple bar chart to compare the increase in expression of each CYP450 isoform in each sample as compared to the negative control (Figure 4).

Processed area ratios results are exported and imported into a Microsoft Excel template for computation of protein level data. Results can be plotted in a simple bar chart to compare the increase in expression of each CYP450 isoform in each sample as compared to the negative control (Figure 4).

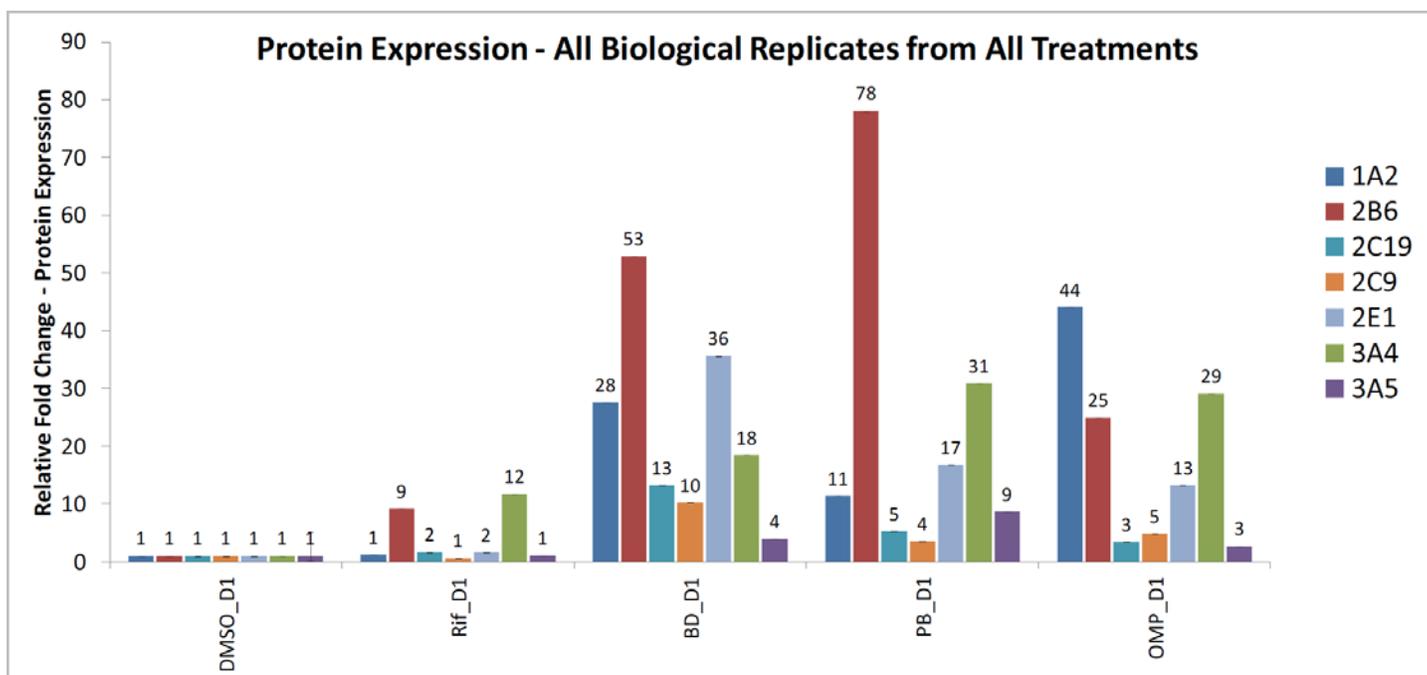


Figure 4. Expression Changes of the Seven CYP Isoforms Measured by LC-MS. Phenobabitol (PB, 1mM) is the prototypical inducer of CYP 2B6 also shows induction of 3A4. Omperasol (OMP, 30µm) is the prototypical inducer of CYP 1A2, rifampicin (RIF, 10µM) is the prototypical inducer of CYP 3A4.

Conclusion

A Cytochrome P450 protein assay kit for human CYP induction analysis is now available for measuring induction of CYPs 1A2, 2B6, 2C9, 2C19, 2E1, 3A4 and 3A5. The kit contains peptide standards, sample preparation reagents and buffers, a column, data acquisition methods, and processing methods and templates for performing protein quantitation using LC-MRM analysis on an SCIEX Triple Quad™ or QTRAP® system. Both the baseline and inducible levels of the CYP isoforms were easily detected by the LC-MRM protein quantitation method. This method allows for a direct measurement of CYP protein levels on the same analytical platform as the enzymatic assays are measured, replacing the need for Western blot analysis.

References

1. Quantitative protein determination for CYP induction via LC-MS/MS, Williamson, BL, Purkayastha, S, Hunter, CL, Nuwaysir, L, Hill, J, Easterwood, I, Hill, J. Proteomics 2011, 11, 33-41
2. MultiQuant™ Software for Protein / Peptide Quantitation: the Gold Standard for Quantitation. SCIEX Technical Note, 2010, Publication 0921210-01
3. To download a trial version of MultiQuant Software please visit: <https://licensing.sciex.com/download/index>
4. Scheduled MRM™ Algorithm Enables Intelligent Use of Retention Times During Multiple Reaction Monitoring. SCIEX Technical Note, Publication 0921010-01.

AB Sciex is doing business as SCIEX.

© 2015 AB Sciex. For research use only. Not for use in diagnostic procedures. The trademarks mentioned herein are the property of the AB Sciex Pte. Ltd. or their respective owners. AB SCIEX™ is being used under license.

RUO-MKT-02-2583-A 07/2015