

CYP450 Protein Assay - Human Induction Kit Quick Reference Card

This quick reference card provides abbreviated procedures you can refer to when using the CYP450 Protein Assay - Human Induction Kit.



Note: For safety and biohazard guidelines, refer to the "Safety" section in the *CYP450 Protein Assay - Human Induction Kit Protocol* (PN 4444550). For every chemical, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Before handling any chemicals, refer to the MSDS provided by the manufacturer and observe all relevant precautions.

Testing the Protocol

It is strongly recommended that, before running samples for the first time, you test the protocol as described in the *CYP450 Protein Assay - Human Induction Kit Protocol* (PN 4444550). Human Liver microsomes and S9 fractions can be obtained for testing from Sigma.

Preparing Sample

Make sure your samples are free of active proteases or high concentrations of detergent or denaturant. If necessary, clean up the samples using acetone precipitation.

The protocol is optimized for 1 mg of protein from microsomal/S9 incubations. Lesser amounts of microsomal/S9 can be used, as long as you maintain a 1:20 trypsin:protein ratio. We do not recommend analyzing less than 100 µg of sample.

Running the Protocol

Follow the procedures shown on the back of this sheet. Modify the procedures if, when testing the protocol, you determine that alternative steps are required for your sample. The protocol description is available at www.sciex.com in the *CYP450 Protein Assay - Human Induction Kit Protocol* (PN 4444550).

Preparing the CYP450 Protein Assay - Human Induction Heavy Peptide Solution

The P450 Peptide Standards vial contains 100 ng (on average) of each of the 12 heavy peptides. Reconstitute with 200 µL of the peptide dilution solvent. The final concentration of each peptide in the solution is approximately 500 pg/µL.


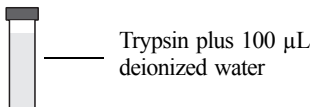

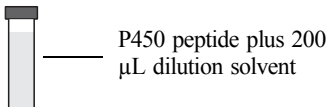
Store the solution at -20 °C until it is ready to use.

Analyzing the CYP450 Protein Assay - Human Induction Sample Mixture

A Triple Quadrupole or QTRAP® System mass spectrometer with analysis software (one of the AB SCIEX 4000 systems (or higher) with MultiQuant™ 1.2 analysis software and the Peptide C18 Column (PN 4445251)) are needed for analysis.

MultiQuant™ Software and Microsoft Excel are required to process the data. Data acquisition methods, processing methods, and a report template can be downloaded from the file called "CYP450 Protein Assay - Human Induction Kit Software Tools.zip" from the following web site:

http://www.absciex.com/mk/get/SOFTWARE_DOWNLOADS

A. Reduce the proteins and block cysteines	B. Digest the proteins with trypsin	C. Add the P450 Peptide Standards solution
<p>1. For each sample, transfer 100 μL microsomes/S9 (10mg/mL) into a tube.</p>  <p>2. Add 5 μL Denaturant.</p> <p>3. Vortex to mix, then spin.</p> <p>4. Add 10 μL Reducing Reagent.</p> <p>5. Vortex to mix, then spin.</p> <p>6. Incubate the tube at 60 °C for 1 hour.</p> <p>7. Add 5 μL Cysteine Blocking Reagent.</p> <p>8. Vortex to mix.</p> <p>9. Incubate the tube at room temperature for 10 minutes.</p>	<p>1. Reconstitute a vial of trypsin with 100 μL of deionized water. This is enough for 10 digestions. Reconstitute more trypsin vials for more samples.</p>  <p>2. Vortex to mix, then spin.</p> <p>3. To each reduced and cysteine-blocked sample that you made in Procedure A, add 100 μL Digestion Buffer.</p>  <p>4. Add 10 μL trypsin solution to each sample.</p> <p>5. Vortex to mix, then spin.</p> <p>6. Trypsin must be used fresh. Discard unused trypsin at the end of each day.</p> <p>7. Incubate the tube(s) at 37 °C overnight (12 to 16 hours).</p>	<p>1. Reconstitute a vial of P450 Peptide Standards with 200 μL of peptide dilution solvent.</p>  <p>2. Add 2 μL P450 Peptide Standards solution to each sample.</p> <p>3. Vortex to mix, then spin.</p> <p>4. Submit all samples for LC-MS analysis.</p> <p>Recommended injection volume is 40 μL.</p>

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

NOTICE TO PURCHASER: PLEASE REFER TO THE CYP450 PROTEIN ASSAY - HUMAN INDUCTION KIT PRODUCT INSERT AND PROTOCOL FOR LIMITED LABEL LICENSE OR DISCLAIMER INFORMATION.

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Technical Resources and Support
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