CYP450 Protein Assay - Human Induction Kit

Reagents for Relative Protein Quantitation of Cytochrome P450 Isoforms — Human 1A2, 2B6, 3A4, and 3A5

Protocol

For Research Use Only
Not for Use in Diagnostics

For Research Use Only. Not for use in diagnostic procedures.

4444550 Rev. B
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Contents

About This Guide

Purpose

The *CYP450 Protein Assay - Human Induction Kit Protocol* provides reference information for the CYP450 Protein Assay - Human Induction Kit including sample preparation, testing and analysis.

Safety information

Note: For general safety information, see this section and Appendix B, "Safety" on page 27. When a hazard symbol and hazard type appear by an instrument hazard, see the "Safety" Appendix for the complete alert on the instrument.

Safety alert words

Four safety alert words appear in our user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

MSDSs

The MSDSs for any chemicals supplied with this kit are available to you free 24 hours a day. For instructions on obtaining MSDSs, see "MSDSs" on page 29.

IMPORTANT! For the MSDSs of chemicals not distributed with this kit contact the chemical manufacturer.

CYP450 Protein Assay - Human Induction Kit

Product information

Purpose of the CYP450 Protein Assay - Human Induction Kit

The CYP450 Protein Assay - Human Induction Kit comprises a set of isotopically enriched peptides useful for performing relative quantitation experiments on four isoforms of the cytochrome P450 family of metabolizing enzymes, CYP 1A2, 2B6, 3A4, and 3A5, by LC/MS using Multiple Reaction Monitoring (MRM). Also supplied are the buffers and reagents needed to perform the enzymatic digestion of the microsomes/S9 fractions prior to LC/MS analysis. Enough buffers and reagents are provided for 100 analyses.

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:

www.absciex.com/support/software/lcmsms

Kit contents

The CYP450 Protein Assay - Human Induction Kit contents are displayed in Table 1.

Three peptides are provided for each of the four isoforms studied, along with reducing/cysteine-blocking reagents and Digestion Buffer. Materials required, but not supplied, are Trypsin with TPCK-Treated Kit (10 pack, P/N 4445250), and Peptide C18 Column (P/N 4445251).

Table 1 ACYP450 Protein Assay - Human Induction Kit Contents

Kit	Quantity of Reagent	Contents (Store at -20 °C)
Denaturant	1 vial, 0.5 mL/vial	Disrupts the hydrogen, hydrophobic, and electrostatic bonds of the proteins. Contains 20% (w/v) n-octyl glucoside (OGS).
Reducing Reagent	1 vial, 1.2 mL/vial	Reduces the disulfide bonds of the proteins. Contains 50 mM tris-(2-carboxyethyl)- phosphine (TCEP).
Cysteine-Blocking Reagent	1 vials, 0.6 mL/vial	Reversibly blocks the cysteine group. Contains 200 mM methyl methane- thiosulfonate (MMTS) in isopropanol.

Kit	Quantity of Reagent	Contents (Store at -20 °C)
Digestion Buffer	6 vials, 2 mL/vial	Buffers the digestion reaction. Contains 0.1 M (Tris [hydroxymethyl] aminomethane hydrochloride) (TRIS), 4 mM Calcium Chloride.
P450 Peptide Standards	1 vial, 100 ng/vial	3 peptides for each isoform: 1A2, 2B6, 3A4, 3A5
Peptide Dilution Solution	1 mL	20% ACN, 0.1% TFA

CYP450 Protein Assay - Human Induction Kit Storage

IMPORTANT! When you receive the shipping container of CYP450 Protein Assay - Human Induction Kit, immediately remove it and store it at –20 °C.

Materials

IMPORTANT! When visually inspecting the reagent vials, the volume of material may appear to be insufficient. During shipment, small volumes of material occasionally become trapped in the cap of the vial. To dislodge the trapped material, allow the vial of reagent to reach room temperature, then briefly centrifuge it. Return the reagents to storage at –20 °C within 2 hours of thawing.

WARNING! CHEMICAL HAZARD. Some of the chemicals provided in your reagent kit may be hazardous. Before handling the reagents, read the material safety data sheets (MSDSs) that accompany your first shipment. Always follow the safety precautions (wearing appropriate protective eyewear, clothing, and gloves, etc.) presented in each MSDS. To receive additional copies of MSDSs at no extra cost, see "MSDSs" on page 29.

For the MSDS of any chemical not found at www.sciex.com, contact the chemical manufacturer. Before handling any chemicals, refer to the MSDS provided by the manufacturer, and observe all relevant precautions.

CYP450 Protein Assay - Human Induction Kit: Materials Not Included

Software

MultiQuant™ software package is available from the AB SCIEX website (see below).

In addition, 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 same web site:

www.absciex.com/support/software/lcmsms

Materials and Equipment

WARNING! CHEMICAL HAZARD. Some of the chemicals referred to in this protocol are not provided with your kit. When using chemicals not provided by or purchased from us, obtain the material safety data sheet directly from the chemical manufacturer.

Table 2 User-supplied materials

Item	Volume or Quantity per Assay
Trypsin with TPCK-Treated Kit (10 pack, P/N 4445250)	One vial contains 500 µg. Each sample requires from 5 µg to 50 µg.
Peptide C18 Column (P/N 4445251)	1 column lasts for at least 200 runs
Disposable gloves	As needed
Test samples Human Liver microsomes and S9 fractions can be obtained for testing from Sigma.	100 µg to 1 mg protein
Autosampler vials	As needed
Tubes	As needed
Deionized water (minimum 18.2 MOhms water, conductivity maximum 0.05 µS/0.05 µMho)	50 mL
Heating block, 60 °C	1
Incubator, 37 °C	1
Bench-top centrifuge	1
Vortexer	1
A Triple Quadrupole or QTRAP® System mass spectrometer with analysis software (one of the AB SCIEX 4000 (or higher) systems with MultiQuant™ 1.2 analysis software and the Peptide C18 Column (PN 4445251)) are needed for analysis.	1
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: **www.absciex.com/support/software/lcmsms**	1

Perform Sample Preparation for Digestion

Review Warnings and Handling Tips

Review the safety warnings in Appendix B, “Safety” on page 27.

IMPORTANT! Slight pipetting variability of small volumes can cause large variability in reagent concentrations and analytical results.

Determine the amount of material

Optimal Protein Concentration is 10 mg/mL. Determine the protein concentration before starting. If the protein concentration is less than 10 mg/mL, then consider concentrating samples in a centrifugal concentrator.

Practice the protocol

If you are running the protocol for the first time, it is strongly recommended that you practice performing the protocol.

Human Liver microsomes and S9 fractions can be obtained and used as a test sample. Process the sample as described in the following procedures.

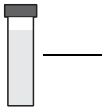
A. Reduce the proteins and block cysteines

1. For each sample, transfer 100 µL microsomes/S9 (10mg/mL) into a tube.

2. Add 5 µL of Denaturant.
3. Vortex to mix, then spin.
4. Add 10 µL of Reducing Reagent.
5. Vortex to mix, then spin.
6. Incubate the tube at 60 °C for 1 hour.
7. Add 5 µL of Cysteine Blocking Reagent.
8. Vortex to mix.
9. Incubate the tube at room temperature for 10 minutes.

B. Digest the proteins with trypsin

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.

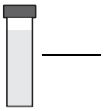


2. Vortex to mix, then spin.
3. To each reduced and cysteine-blocked sample that you made in Procedure A, add 100 µL of Digestion Buffer.

4. Add 10 µL of trypsin solution to each sample.
5. Vortex to mix, then spin.
6. Trypsin must be used fresh. Discard unused trypsin at the end of each day.
7. Incubate the tube(s) at 37 °C overnight (12 to 16 hours).

C. Add the P450 Peptide Standards solution

1. Reconstitute a vial of P450 Peptide Standards with 200 µL of peptide dilution solvent.



2. Add 2 µL of P450 Peptide Standards solution to each sample.
3. Vortex to mix, then spin.
4. Submit all samples for LC/MS analysis.
   The recommended injection volume is 40 µL.

LC/MS/MS MRM Analysis

Initial LC/MS/MS MRM Settings

The suggested LC/MS/MS MRM settings presented here are recommended for analyzing samples with the AB SCIEX 4000 QTRAP® System or API 4000TM LC/MS/MS System. These settings provide a starting point for developing the optimal settings for your samples and system.

To order or download PDF documents helpful when using the systems noted above (such as system user guides and tutorials or technical notes), see:

www.absciex.com/support

Importing MRM Method

This procedure provides a starting point for developing the optimal settings for your samples and system.

1. Open the Excel file named CYP 450 MRM.xls.
2. Copy and paste the contents directly into the MRM page of a new acquisition file.
3. Ensure the MS parameters are setup as described in Table 3, “Suggested MRM Settings for the 4000 QTRAP System”.
4. Setup the LC parameters as shown in Table 5, “Suggested gradient time and percent mobile phase B for LC/MS”.

Table 3 Suggested MRM Settings for the 4000 QTRAP System

Peptide	Q1 Mass (amu)	Q3 Mass (amu)	DP	CE	CXP
CYP 1A2					
Peptide 1 - Light	432.7	636.4	55	20	20
	432.7	535.3	55	20	20
	432.7	294.2	55	30	19
Peptide 1 - Heavy	436.7	644.4	55	20	20
	436.7	543.3	55	20	20
	436.7	302.2	55	30	19
Peptide 2 - Light	528.7	501.2	70	26	20

Peptide	Q1 Mass (amu)	Q3 Mass (amu)	DP	CE	CXP
	528.7	614.4	70	27	20
	528.7	727.4	70	25	20
Peptide 2 - Heavy	532.7	509.2	70	26	20
	532.7	622.4	70	27	20
	532.7	735.4	70	25	20
Peptide 3 - Light	536.3	795.4	70	26	18
	536.3	584.3	70	32	18
	536.3	698.4	70	35	18
Peptide 3 - Heavy	541.3	805.4	70	26	18
	541.3	594.3	70	32	18
	541.3	708.4	70	35	18
CYP 2B6					
Peptide 1 - Light	548.3	780.4	60	35	15
	548.3	681.3	60	23	15
	548.3	566.4	60	23	15
Peptide 1 - Heavy	553.4	576.4	60	35	15
	553.4	691.3	60	23	15
	553.4	921.4	60	23	15

Peptide	Q1 Mass (amu)	Q3 Mass (amu)	DP	CE	CXP
Peptide 2 - Light	421.2	508.2	70	23	15
	421.2	607.3	70	22	15
	421.2	694.3	70	24	15
Peptide 2 - Heavy	426.2	518.4	70	23	15
	426.2	617.3	70	22	15
	426.2	704.4	70	24	15
Peptide 3 - Light	479.2	499.3	55	22	15
	479.2	614.3	55	22	15
	479.2	727.4	55	22	15
Peptide 3 - Heavy	483.5	507.3	55	22	15
	483.5	622.3	55	22	15
	483.5	735.4	55	22	15
CYP 3A4					
Peptide 1 - Light	439.7	532.3	50	21	15
	439.7	549.3	50	21	15
	439.7	650.5	50	21	15
Peptide 1 - Heavy	444.9	542.3	50	21	15
	444.9	559.3	50	21	15
	444.9	660.4	50	21	15
Peptide 2 - Light	564.3	789.5	60	23	15
	564.3	745.9	60	23	15
	564.3	683.4	60	23	15
Peptide 2 - Heavy	567.3	793.5	60	23	15
	567.3	750.0	60	23	15
	567.3	693.4	60	23	15

Peptide	Q1 Mass (amu)	Q3 Mass (amu)	DP	CE	CXP
Peptide 3 - Light	798.4	819.4	80	28	19
	798.4	932.5	80	29	19
	798.4	1003.4	80	29	19
Peptide 3 - Heavy	801.0	827.4	80	28	19
	801.0	940.5	80	29	19
	801.0	1011.4	80	29	19
CYP 3A5					
Peptide 1 - Light	469.5	494.3	55	25	12
	469.5	608.3	55	21	15
	469.5	721.4	55	19	15
Peptide 1 - Heavy	473.5	502.4	55	25	12
	473.5	616.4	55	21	15
	473.5	729.5	55	19	15
Peptide 2 - Light	468.3	735.5	55	20	15
	468.3	678.5	55	22	15
	468.3	581.4	55	28	15
Peptide 2 - Heavy	472.3	589.3	55	28	15
	472.3	686.4	55	22	15
	472.3	743.5	55	20	15
Peptide 3 - Light	589.1	646.5	60	20	15
	589.1	696.0	60	21	15
	589.1	745.5	60	19	15
Peptide 3 - Heavy	592.0	650.5	60	20	15
	592.0	700.0	60	21	15
	592.0	749.5	60	19	15

Table 4 Suggested mobile phases A and B

Compound	Mobile Phase A	Mobile Phase B
Deionized water	98%	2%
Acetonitrile, HPLC-grade	2%	98%
Formic acid	0.1%	0.1%

Table 5 Suggested gradient time and percent mobile phase B for LC/MS

Time	Flow Rate (µL/min)	%B
0.1	700	5
10	700	50
15	700	90
16	700	90
17	700	5
20	700	5

Table 6 Suggested source parameters

4000 QTRAP® System Parameters	Setting
CUR	25
CAD	High
IS	4000
TEM	650
GS1	55
GS2	60
ihe	ON

Initial Testing of Method

In order to determine the retention time of the eluting peptides and set up a Scheduled MRM™ Algorithm method, a working solution of the P450 Peptide Standards mix should be made. From the stock solution (500 pg/µL) make up a 20 pg/µL solution using the supplied peptide dilution buffer. A 10 µL injection of this solution will result in 200 pg on column, which should give good signal to noise MS peaks. An example chromatogram is shown in Figure 1 on page 20 for the analysis of 200 pg of the P450 peptide on a API 4000 system. Typical retention times are shown in Table 7 on page 20.

Running of Test Sample

Human Liver microsomes and S9 fractions can be obtained for testing from Sigma and used as a test sample. Process the sample as described in "Perform Sample Preparation for Digestion" on page 11.

Transfer 200 µL of Digest Buffer to a fresh autosampler vial and add 2 µL of the P450 Peptide Standards solution. Use 40 µL per injection and run the sample in triplicate. Follow the instructions for processing the data and ensure endogenous and heavy peptide are observed.

Figure 1 LC/MS of the peptide standards

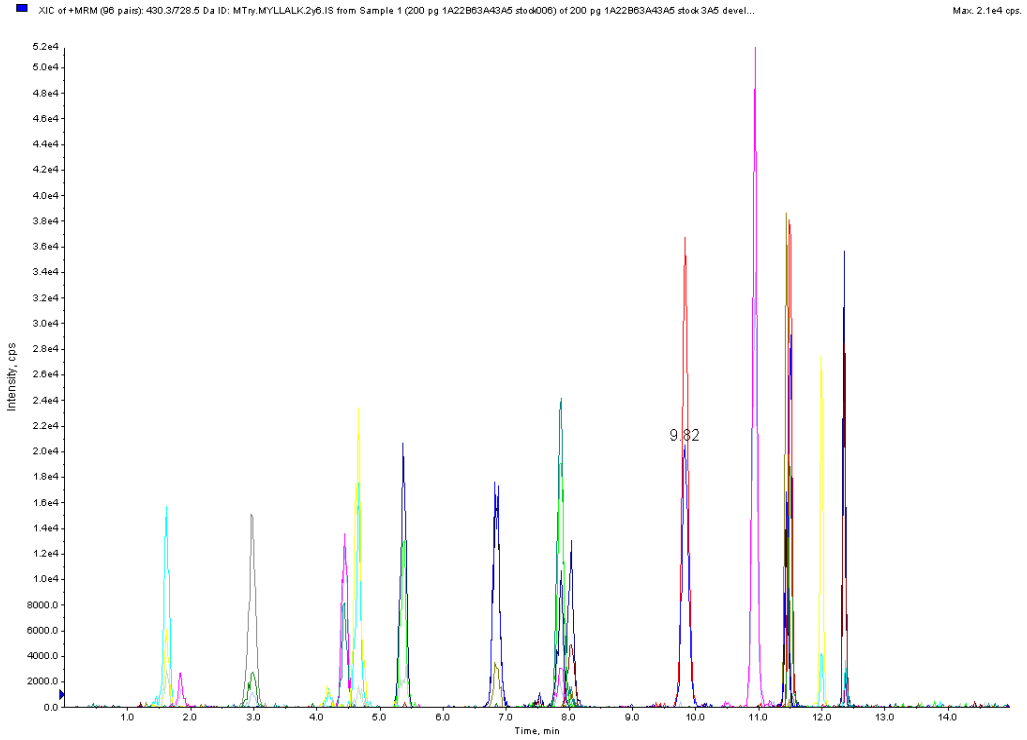


Table 7 Typical LC retention times

Peptide	Typical Retention Time
CYP 1A2	
Peptide 1 - Light	7.5
Peptide 2 - Light	2.4
Peptide 3 - Light	4.0
CYP 2B6	
Peptide 1 - Light	11.1
Peptide 2 - Light	3.9
Peptide 3 - Light	1.2
CYP 3A4	
Peptide 1 - Light	4.5
Peptide 2 - Light	11.3
Peptide 3 - Light	12.3
CYP 3A5	
Peptide 1 - Light	6.9

Peptide	Typical Retention Time
Peptide 2 - Light	7.8
Peptide 3 - Light	7.8

Analyzing Data

Open MultiQuant™ software and select “Quantitate New Data” (see MultiQuant™ manual for details). Select your induction data set and choose to create a new method. Create a name for your new quantitation method and then select a data file to use to build your quantitation method. An example of typical integration parameters are shown in “Typical integration parameters” on page 22.

Process samples with the finished MultiQuant™ method and ensure each peak is correctly integrated.

Edit column settings and ensure the “Area ratio” column is selected.

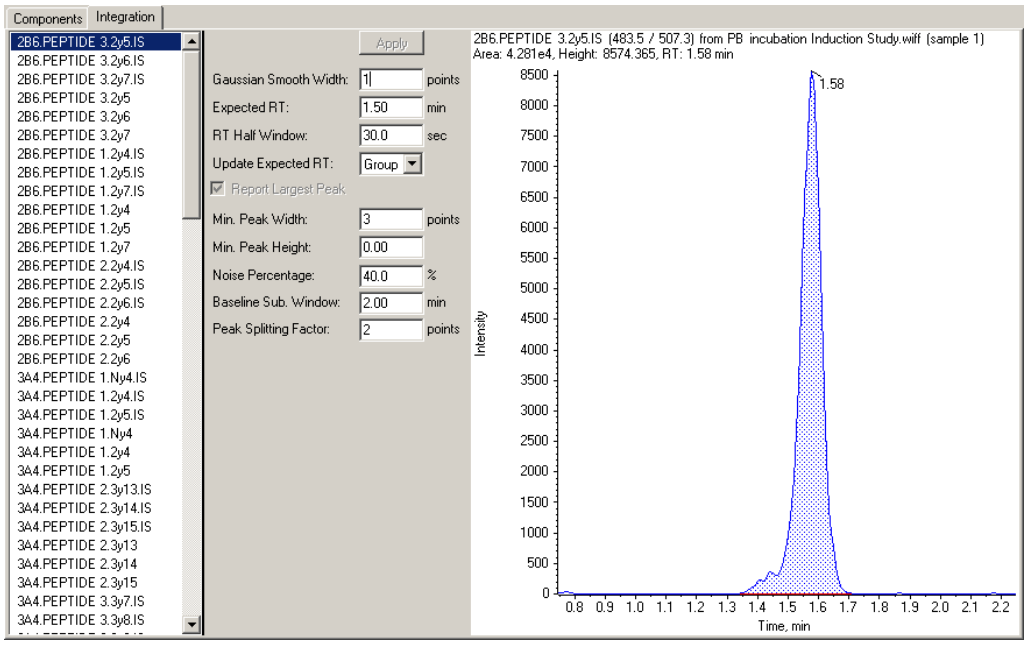
Now the entire table can be copied into Microsoft Excel and the area ratio column can be plotted out for the Peptides and isoforms of each sample.

Figure 2 Typical MultiQuant method settings

Experiment: +MRM (72 transitions)

Row	IS	Name	Group	IS Name	Q1 / Q3	RT (min)
1	☑	2B6.PEPTIDE 3.2y5.IS	2B6.PEPTIDE 3		483.5 / 507.3	1.50
2	☑	2B6.PEPTIDE 3.2y6.IS	2B6.PEPTIDE 3		483.5 / 622.3	1.50
3	☑	2B6.PEPTIDE 3.2y7.IS	2B6.PEPTIDE 3		483.5 / 735.4	1.50
4	☐	2B6.PEPTIDE 3.2y5	2B6.PEPTIDE 3	2B6.PEPTIDE 3.2y5.IS	479.2 / 499.3	1.50
5	☐	2B6.PEPTIDE 3.2y6	2B6.PEPTIDE 3	2B6.PEPTIDE 3.2y6.IS	479.2 / 614.3	1.50
6	☐	2B6.PEPTIDE 3.2y7	2B6.PEPTIDE 3	2B6.PEPTIDE 3.2y7.IS	479.2 / 727.4	1.50
7	☑	2B6.PEPTIDE 1.2y4.IS	2B6.PEPTIDE 1		553.5 / 576.4	8.30
8	☑	2B6.PEPTIDE 1.2y5.IS	2B6.PEPTIDE 1		553.5 / 691.3	8.30
9	☑	2B6.PEPTIDE 1.2y7.IS	2B6.PEPTIDE 1		553.5 / 921.4	8.30
10	☐	2B6.PEPTIDE 1.2y4	2B6.PEPTIDE 1	2B6.PEPTIDE 1.2y4.IS	548.3 / 566.3	8.30
11	☐	2B6.PEPTIDE 1.2y5	2B6.PEPTIDE 1	2B6.PEPTIDE 1.2y5.IS	548.3 / 681.3	8.30
12	☐	2B6.PEPTIDE 1.2y7	2B6.PEPTIDE 1	2B6.PEPTIDE 1.2y7.IS	548.3 / 911.4	8.30
13	☑	2B6.PEPTIDE 2.2y4.IS	2B6.PEPTIDE 2		426.2 / 518.4	3.70
14	☑	2B6.PEPTIDE 2.2y5.IS	2B6.PEPTIDE 2		426.2 / 617.3	3.70
15	☑	2B6.PEPTIDE 2.2y6.IS	2B6.PEPTIDE 2		426.2 / 704.4	3.70
16	☐	2B6.PEPTIDE 2.2y4	2B6.PEPTIDE 2	2B6.PEPTIDE 2.2y4.IS	421.2 / 508.4	3.70
17	☐	2B6.PEPTIDE 2.2y5	2B6.PEPTIDE 2	2B6.PEPTIDE 2.2y5.IS	421.2 / 607.3	3.70
18	☐	2B6.PEPTIDE 2.2y6	2B6.PEPTIDE 2	2B6.PEPTIDE 2.2y6.IS	421.2 / 694.4	3.70
19	☑	3A4.PEPTIDE 1.Ny4.IS	3A4.PEPTIDE 1		444.9 / 542.3	4.40
20	☑	3A4.PEPTIDE 1.2y4.IS	3A4.PEPTIDE 1		444.9 / 559.3	4.40
21	☑	3A4.PEPTIDE 1.2y5.IS	3A4.PEPTIDE 1		444.9 / 660.4	4.40

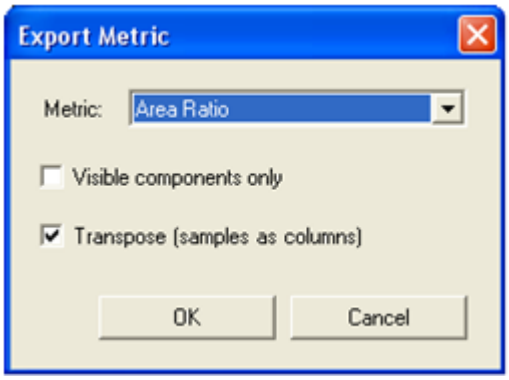
Figure 3 Typical integration parameters



Cytochrome P450 MRM Induction Assay Analysis

This procedure provides instructions for performing an assay data analysis.

1. Export data from MultiQuant.
   a. Go to File > Export > Results Table - Metric.
   The Export Metric window opens.



   b. Export both the Area Ratio and the Area metric tables. Be sure to select Transpose (samples as columns). Click OK.

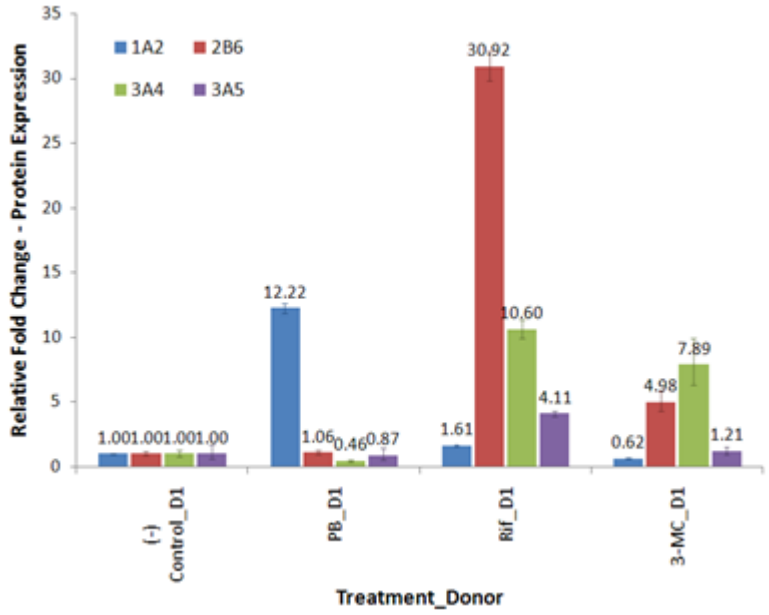
2. Enter text data into the Excel template by pasting MultiQuant exports into corresponding 'INPUT-...' tabs.
   a. Open the text files with Excel.
   b. Select and copy the text (Ctrl-A, Ctrl-C).
   c. Place the cursor in the top left corner (square A1) of the CYP450 template.
   d. Right-click and select Paste Special > Values.

   The first three rows are for headers, the actual data starts on row 4. There is only one column for row headers.

3. Assign metadata by adjusting the information in columns E through I in the 'INPUT - Metadata Association' tab.

4. Press F-9 to perform calculation and generate a graph of the data.
   Detailed instructions are contained in the P450 Analysis Template.

Figure 4 Graph of Cytochrome P450 MRM Induction Assay Analysis



Ordering Information

How to order

Materials that are required, but are not supplied in this kit (See Table 2, "User-supplied materials" on page 9) are available from:

www.sciex.com

Safety

This appendix covers:

Chemical safety

General chemical safety

Chemical hazard warning

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING! CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See “About MSDSs” on page 29.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer’s cleanup procedures as recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

MSDSs

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to new customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

You can obtain the MSDS for any chemical supplied with this kit at **www.sciex.com/msds**.

Note: For the MSDSs of chemicals not distributed with this kit, contact the appropriate chemical manufacturer.

Chemical waste safety

Chemical waste hazards

CAUTION! HAZARDOUS WASTE. Refer to Material Safety Data Sheets and local regulations for handling and disposal.

WARNING! CHEMICAL WASTE HAZARD. Wastes produced by our instruments are potentially hazardous and can cause injury, illness, or death.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.

- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

  IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

General biohazard

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **www.access.gpo.gov/ nara/cfr/waisidx_01/ 29cfr1910a_01.html**).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

www.cdc.gov

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AB Sciex Pte. Ltd
110 Marsh Road
Foster City, CA 94404 USA
1 877-740-2129
www.sciex.com

Technical Resources and Support
For the latest technical resources and support information for all locations, please refer to our Web site at:
www.sciex.com/support