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Silensomes™ CYP Phenotyping Kit

Technical Information & Instructions

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I. Introduction

Silensomes™ products were developed to overcome the disadvantages of the current CYP450 phenotyping methodologies. Silensomes™ CYP Phenotyping Kits consist of human pooled liver microsomes (HLM) that are chemically and irreversibly inactivated for one specific CYP450 activity (1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4) and control HLM that were treated the same way without inactivation. Silensomes™ HLM and Control Silensomes™ HLM are handled in the same manner as conventional HLM and are ready-to-use for phenotyping assays. Each Silensomes™ HLM batch exhibits a high specificity and targeted CYP inhibition (>80 %). This protocol outlines instructions for using Silensomes™ CYP Phenotyping Kits to perform reaction phenotyping of new chemical entities. As these test chemicals have highly variable chemical properties, it is important to empirically determine optimal conditions for any new test compound.

II. Product information

NOTE: All Silensomes™ HLM Kit vials contain 150 µL of Silensomes™ HLM. Protein concentration is 20 mg/ml.

Product Description	Cat. No.	Kit Contents
Master Control Silensomes™ HLM	SIL000	1 X Master Control Silensomes™ HLM
Silensomes™ CYP3A4 Kit with Control	SIL200K	1 X Silensomes™ CYP3A4 HLM 1 X Master Control Silensomes™ HLM
Silensomes™ CYP1A2 Kit with Control	SIL210K	1 X Silensomes™ CYP1A2 HLM 1 X Master Control Silensomes™ HLM
Silensomes™ CYP2A6 Kit with Control	SIL220K	1 X Silensomes™ CYP2A6 HLM 1 X Master Control Silensomes™ HLM
Silensomes™ CYP2B6 Kit with Control	SIL230K	1 X Silensomes™ CYP2B6 HLM

		1 X Master Control Silensomes™ HLM
Silensomes™ CYP2D6 Kit with Control	SIL240K	1 X Silensomes™ CYP2D6 HLM 1 X Master Control Silensomes™ HLM
Silensomes™ CYP2C8 Kit with Control	SIL250K	1 X Silensomes™ CYP2C8 HLM 1 X Master Control Silensomes™ HLM
Silensomes™ CYP2C9 Kit with Control	SIL260K	1 X Silensomes™ CYP2C9 HLM 1 X Master Control Silensomes™ HLM
Silensomes™ CYP2C19 Kit with Control	SIL270K	1 X Silensomes™ CYP2C19 HLM 1 X Master Control Silensomes™ HLM
Silensomes™ CYP Panel Phenotyping Kit	SIL-MCPNL	1 X Silensomes™ CYP3A4 HLM 1 X Silensomes™ CYP1A2 HLM 1 X Silensomes™ CYP2A6 HLM 1 X Silensomes™ CYP2B6 HLM 1 X Silensomes™ CYP2D6 HLM 1 X Silensomes™ CYP2C8 HLM 1 X Silensomes™ CYP2C9 HLM 1 X Master Control Silensomes™ HLM

For answers to frequently asked questions regarding these products, please visit our FAQ Database:
www.lonza.com/faq

For citations referencing the use of these products, please visit our Citations Database:
www.lonza.com/citations

III. Required Reagents and Materials

Buffer	0.1M phosphate buffer pH 7.4, Sigma P5244 or equivalent
Supplement	1M MgCl ₂ solution, Fisher AM9530G or equivalent
Cofactor	10mM NADPH stock solution
Stop Solution	Acetonitrile, methanol, 0.15M Phosphoric Acid or other suitable organic solvent
Wet Ice Bath	Wet ice or other constant temperature vessel for holding samples at 4°C prior to reaction
Water Bath	Water bath or other vessel capable of maintaining samples at 37°C
Microfuge	Refrigerated microfuge capable of 3,500 x g spin speed
Culture Tubes	5 ml glass tubes, Pyrex 99445-12 or equivalent
96-well Plates	Lonza Cat. No. 25-340 or equivalent

IV. Unpacking and Storage Instructions

Check all containers for leakage or breakage. Store all Silensomes™ Cyp Phenotyping Kit Vials at -70°C immediately upon receipt.

V. General Product Information

The number of reaction incubations that can be conducted with one Silensomes™ CYP Phenotyping Kit will depend on the protein concentration selected. Examples of different protein concentrations and sample numbers for phenotyping assays depend on the rate of metabolism.

Expected Rate of Metabolism (fast, medium, slow)	Selected Protein Concentration for Assay (mg/mL)	Number of Assays per Vial
Fast	0.3	100 (2.5 mL, 50 µL/sample)
Medium	1	30 (2.5 mL, 50 µL/sample)
Slow	2	15 (1.25 mL, 50 µL/sample)

- If the rate of the metabolism, the incubation time and protein concentration linearities are not known for the test compounds, the linearity tests should be conducted in conventional HLM and the optimum conditions chosen for the assays prior to starting experiment with the Silensomes™ Kit
- Incubation conditions with Silensomes™ HLM and Control Silensomes™ HLM must follow linearity, *i.e.*, metabolite formation is directly proportional to time and protein concentration
- 10mM NADPH is required as an enzymatic co-factor and serves to start the reaction

VI. Protocol Selection

Two experimental approaches for using Silensomes™ CYP Phenotyping Kits to determine the fraction metabolized by each P450 are outlined below.

NOTE: If the test compound needs to be dissolved in an intermediate solvent, care must be taken with respect to the solvent type (e.g. DMSO) and concentration as some CYP450s are inhibited by solvents. Use the lowest possible solvent concentration.

Protocol 1: Six Incubation Time Points

Incubation times (minutes)	0,10, 20, 30, 45
Replicates	N = 1 well for assays
Incubation volume	450 µL
Microsomal concentration	1 mg/mL or optimal concentration based on HLM linearity

Protocol 2: Single Incubation Time Point

Incubation time	One incubation time in the linear portion of the depletion profile using HLM
Replicates	N = 3 wells for assays
Incubation volume	100 µL
Microsomal concentration	1 mg/mL or an optimal concentration based on HLM linearity

NOTE: When using one microsomal concentration, select a concentration that is below the K_m for enzyme interactions with the test compound and considers the limit of solubility. Ideally, a pharmacologically relevant concentration of the test compound is tested. If K_m is unknown, this should be determined using conventional microsomes prior to using Silensomes™ HLMs for phenotyping studies.

VII. Thawing of Silensomes™ HLM and Control Silensomes™ HLM

Thaw each vial in the Silensomes™ CYP Phenotyping Kit slowly on ice and maintain at ~4°C on ice until use.

NOTE: Silensomes™ HLM and Control Silensomes™ HLM can withstand up to 10 freeze-thaw cycles without loss of CYP450 activities. Silensomes™ HLM CYP450 enzymatic activities are stable for up to 5 years when stored at -70°C.

NOTE: After dispensing the required volume of Silensomes™ HLM and Control Silensomes™ HLM for your studies, maintain the remaining volume on ice, re-freeze as quickly as possible and store at -70°C.

VIII. Protocol Initiation

1. Prepare the test compound and NADPH cofactor stock solution and place them on ice.
2. Supplement the 100 ml bottle of phosphate buffer with 0.5 ml of the 1M MgCl₂ solution (to achieve a working buffer solution with a final MgCl₂ concentration of 5 mM) and place the supplemented buffer on ice.

3. Thaw Silensomes™ HLM according to the instructions in Section VII.
4. Pre-incubate Silensomes™ HLM and Control Silensomes™ HLM, supplemented buffer and test compound in a 37°C in water bath for 5 minutes.
5. Combine all components *except NADPH* in tubes/well-plates as described in Table 1 or Table 2 per protocol selection and mix by gently rocking/shaking the tubes/plate.
6. Initiate the reaction by adding NADPH to the tubes or 96-well plate as described in Table 1 or Table 2 per protocol selection and mix by gently rocking/shaking the tubes/plate.

Table 1

Six Incubation Time Points	Final Microsomal Protein Concentration	
	1mg/ml	0.3mg/ml
Silensomes™ HLM or Control Silensomes™ HLM	22.5 µl	6.75 µl
Supplemented Buffer	382.54 µl	398.25 µl
10mM NADPH Solution	45 µl	45 µl
Total Reaction Volume	450 µl	450 µl

Table 2

Single Incubation Time Point	Final Microsomal Protein Concentration	
	1mg/ml	0.3mg/ml
Silensomes™ HLM or Control Silensomes™ HLM	5 µl	1.5 µl
Supplemented Buffer	85 µl	88.5 µl
10mM NADPH	10 µl	10 µl
Total Reaction Volume	100 µl	100 µl

7. Incubate at 37°C for the appropriate time period(s) according to the protocol selected (see Section VI).
8. At each incubation time point, stop the reaction using an equal portion of organic solvent and briefly shake.
 - a. If using Protocol 1 (six time points), remove 50 µl of reaction volume and add to a new tube or well containing 50 µl of organic solvent stop solution.
9. If using Protocol 2 (single time point), add stop solution in equal volume directly to the reaction tube or well.
10. Store tubes/well-plates at -4°C for 1 h to allow protein precipitation to occur.
11. Centrifuge the tubes/well-plates for 15 min at 3500 x g, 4°C.
12. Remove the supernatant and analyse immediately or store at -80°C (for a maximum of 6 months) until analysis by HPLC-MS/MS.

IX. Important Equations for CYP Phenotyping Using Silensomes™ HLM

Equation 1: In vitro Clearance: The *in vitro* intrinsic clearance (Cl_{int} , mL/min*g protein) can be determined by measuring the substrate depletion (representing total metabolites), using the following equation:

$$Cl_{int} = \frac{\text{Slope} \times \text{Vol}}{\text{prot}}$$

Equation 2: CYP Contribution (fm): The CYP contribution to the metabolism of the test compound is estimated by calculating the ratio of the intrinsic clearance values obtained for Silensomes™ HLM (Cl_{int} SiL, mL/min.g protein) and for Control Silensomes™ HLM (Cl_{int} cSiL, mL/min*g protein).

$$fm = 1 - \left(\frac{Cl_{int} \text{ SiL}}{Cl_{int} \text{ cSiL}} \right)$$

Equation 3: CYP Contribution: The relative contribution of the CYP of interest is estimated using the following equation:

$$\text{Contribution} = \left(1 - \left(\frac{Cl_{int} \text{ SiL}}{Cl_{int} \text{ cSiL}} \right) \right) \times 100$$

X. Product Warranty

Lonza guarantees the performance of Silensomes™ only if appropriate buffers and reagents are used exclusively and the recommended storage and use protocols are followed. Any modifications made to the recommended systems including the use of alternative buffers, reagents or protocols, will void performance guarantees. If you need assistance in selecting the appropriate media, reagents, or protocol, please contact Lonza Scientific Support (see details on Page 1).

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* diagnostic procedures.

WARNING: LONZA SILENSOMES™ HLM PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA-approved method for the presence of HIV-1, hepatitis B virus and hepatitis C virus. All human-sourced products should be handled at the biological safety level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH manual, [Biosafety in Microbiological and Biomedical Laboratories, 5th ed.](#) If you require further information, please contact your site safety officer or Scientific Support.

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