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CypExpress Protocol

Procedural Notes

- A starting substrate concentration of 500 µM should be used. The concentration that produces the highest level of metabolite varies for each compound and can be optimized using the Pilot Procedure.
- Many drugs are poorly soluble in water and can be difficult to dissolve in buffer. This can be remedied by preparing a concentrated solution of the compound in dimethylsulfoxide or *N,N*-dimethylformamide and adding it to the CypExpress/buffer suspension.
- Alcohols should never be used in any CypExpress reactions.
- A 20 mg/mL suspension concentration of CypExpress in buffer is recommended.

Materials

- Allow all reagents to warm to room temperature before starting -

- CypExpress powder
- 100 mM, pH 7.4 potassium phosphate buffer containing 5.0 mM glucose-6-phosphate (G6P) and 2.0 mM nicotinamide adenine dinucleotide phosphate, sodium salt ($NADP^+$)
- Concentrated substrate solution in dimethylsulfoxide

Procedure for a Two Milliliter Reaction

1. Place 40 mg of CypExpress powder into a test tube with a stir bar. A 16 mm by 125 mm tube works well for this.

2. Add 2.0 mL of buffer containing G6P and $NADP^+$ to the powder and begin stirring to make a suspension.
3. Add the concentrated substrate stock to achieve a 500 µM concentration. For example, adding 2.0 µL of a concentrated 0.5 M testosterone DMSO stock solution to 2.0 mL of buffer gives a final concentration of 500 µM testosterone.
4. Stir the uncovered tube at 37°C fast enough to create a vortex.
5. Allow the reaction to proceed for four hours.
6. Centrifuge the sample at 6,000×*g* for 10 minutes at room temperature.
7. Remove the supernatant and re-suspend the pellet in 1.0 mL of acetone.
8. Centrifuge, remove the supernatant and combine it with the first supernatant sample.
9. Remove the water and acetone to give the metabolites and starting material for separation and analysis.

If you have any questions, please contact us:
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