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Product Specification Sheet

Description: CypExpress™ 2D6
Product Number: CE2D6.10
Aliquot: 10 Grams
Lot Number: ce2d6.2
Storage: -80°C

DESCRIPTION: CypExpress™ 2D6 is a permeabilized and stabilized dried yeast powder preparation containing recombinant human CYP 2D6 and recombinant human P450 NADPH oxidoreductase.

ACTIVITY: A reaction mixture containing 1 mM dextromethorphan and 100 mg/mL of CypExpress™ 2D6 will produce 268.8 uM dextrorphan in 4 hours at 30°C under the conditions described.

THAWING: It is important to avoid water adsorption during thawing. Remove the CypExpress™ vial from storage and warm to room temperature prior to opening. Return unused powder to -80°C.

STORAGE: Store at -80°C. Although CypExpress™ preparations can be warmed to room temperature multiple times, it is best to aliquot as needed to avoid multiple freeze/thaw cycles.

SUGGESTED PROTOCOLS: The basic protocols below are intended as a starting point that can be optimized by an experienced user for specific applications and samples.

Rapid Screening of Metabolites in 24-Well Microtiter Plates

All reactions are in 50 mM potassium phosphate buffer (KPi), pH 7.5. Final substrate concentration is at 250-500 μM. Up to 2% v/v DMSO/DMF is compatible with CypExpress™.

1. Remove the CypExpress™ frozen powder and let it thaw for 20-30 min at room temperature. Prepare a 100 mg/mL uniform suspension of CypExpress™ in KPi to the extent required. Return the CypExpress™ powder to the freezer.
2. **OPTIONAL:** Prewash the powder to minimize background (see steps 3 and 4 of the scale-up protocol on the following page. This step is especially important if only HPLC (vs HPLC/MS) is utilized for detection. If washed, some loss of activity (~20%) will be observed, but can be avoided by adding NAD+ and glucose 6-phosphate to the reaction mixture..
3. Dispense a final volume of 200μL of powder and the substrate as recommended in a 24-well plate (if 1 mL reaction is preferred, use 20 x 150 mm glass test-tube for consistent product formation and incubate at ~ 45° angle for better aeration in an orbital shaker).
4. Incubate the microtiter plate at 30° C on an orbital shaker at 600 rpm for 3-4 hours. Note: Well to well variation in metabolite formation occurs in microtiter plates due to aeration dynamics. For quantitation, reactions should be performed in triplicate.
5. Stop the reaction by adding HPLC mobile phase in the ratio of 2:3 (v/v, e.g., 150 μL mobile phase for 100 μL reaction mixture).
6. Let it sit for ~30 min at room temperature for complete precipitation.

7. Centrifuge the reaction mixture at 14,000 x *g* for 10 min at 20° C.
8. Filter the supernatant through a 0.22 μm filter.
9. Inject 10-20 μL of the filtered sample into HPLC or LC-MS for substrate disappearance & metabolite identification with appropriate standards, preferably using MS with selective ion monitoring.

Scaling-up for Metabolite Isolation (5+ mg)

It is recommended that the optimum substrate concentration (0- 500/1000 μM) and reaction time be established in 24-well microplates or 1 mL tubes using the Rapid Screening conditions above at pH 7.5.

1. Remove the CypExpress™ frozen powder from the freezer and let it thaw at room temperature for 20-30 min.
2. Weigh 20 g of powder and add it to 320 mL of 50 mM KP_i, pH 7.5 in a large baffled conical flask (1.8 – 2.4 L) to wash the powder. This washing step is extremely important to get a clean HPLC separation of the metabolite and to eliminate background. For reactions greater than 200mL, adjust the powder and washing volume proportionally.
3. Incubate the uniform cell suspension at 30° C for approximately 20 min, at 175 rpm in an orbital shaker. Then centrifuge the suspension at 6,000 x *g* for 4 min at 4° C.
4. Resuspend washed in 200 mL of the buffer
5. Add 12 mM D-glucose-6-phosphate-Na (G6P & optimum substrate concentration).
6. Incubate the reaction mixture in a large baffled conical flask (1.8 – 2.4 L) at 225 rpm, 30° C to ensure adequate aeration. The optimum reaction time should be established as described above.
7. Centrifuge the suspension at 6000 x *g* for 4 min at 4° C. Save the supernatant from the 1st cycle. Take 500 μL for metabolite analysis after addition of HPLC mobile phase for precipitation.
8. For 2nd reaction cycle, resuspend the pellet in the buffer and add 12 mM G6P (total reaction volume 200 mL). Additional substrate is not required.
9. Incubate the reaction mixture for the optimum time.
10. Take 500 μL of the suspension, and 500 uL of reaction mixture without cells (after centrifugation of 1.0 mL reaction mixture) separately in two micro centrifuge tubes. Add 750 uL of the HPLC mobile phase and let it sit for 30 min for complete precipitation. Spin both samples at 14000 x *g* for 10 min at 20° C. Filter both supernatants and analyze for metabolite formation in both samples.
11. If supernatants from the two cycles account for at least 80% of the metabolite, proceed with combining the supernatants from both cycles for metabolite isolation as in Step 10 after precipitating the macromolecules with the HPLC mobile phase.