

Protocol for Hydrolysis Assay by Carboxylesterases (CEs) Enzyme

Preparation:

1. Prepare an ice bucket.
2. Prepare stock solution of irinotecan in DMSO (or DMSO:EtOH) at 10 mM and dilute the stock solution into 1 mM using DMSO (or DMSO:EtOH) as the working solution. Put the irinotecan working solution on ice.
3. Prepare enzyme working solution (1 mg/ml) from the stock solution (dilute the crude fecal enzymes with KPI to afford a working solution at 1 mg/mL) on ice.
4. Prepare the quenching solution (6% formic acid in acetonitrile).
5. Prepare two sets of 1.7-mL microcentrifuge tubes: one set is for incubation, the other is for sample collection. Label the tubes appropriately.
6. Put the incubation tubes on ice and put the sample tubes on a microcentrifuge tube rack. Using different color tubes for different enzymes or drugs.

Steps:

1. Add 25 uL of the quenching solution into each sample tube on the rack.
2. Add enzyme working solution into the incubation tubes on ice (following the volume in the table, you can adjust the volume of the working solution and KPI buffer to make the final volume consistent).
3. Add KPI buffer into the incubation tubes on ice.
4. Add irinotecan working solution into the incubation tubes on ice.
5. Put the incubation tubes on water bath (37°C).
6. Take 100 uL of solution from the incubation tubes at time 0 into the sample collection tubes, in which the quenching solution (25 uL) has been added as described above.
7. Take 100 uL of solution from the incubation tubes at 0.5, 1, 2, and 4 hours into the sample collection tubes, in which the quenching solution has been added as described above.
8. Vortex the solution and centrifuge at 14,000rpm, 4°C for 15mins.
10. Collect about 100 uL of the supernatant for UPLC injection.
11. Adjust irinotecan working solution volume and the KPI volume to conduct reaction at different concentrations.

For 10 uM drug concentration

Fold	Enzyme working solution (1 mg/mL)	KPI	Irinotecan working solution (1 mM)	Total volume	Final drug concentration
2	100 uL	98 µL	2 µL	200 µL	10 µM
6	6 µL	588 µL	6 µL	600 µL	10 µM

0.5 mg/mL, 30 min.

500 ug/mL, 30 min

For 5 uM drug concentration

Fold	Enzyme working solution (1 mg/mL)	KPI	Irinotecan working solution (1 mM)	Total volume	Final drug concentration
2	2	197 µL	1 µL	200 µL	5 µM
6	6 µL	591 µL	3 µL	600 µL	5 µM

For 20 uM drug concentration

Fold	Enzyme working solution (1 mg/mL)	KPI	Irinotecan working solution (1 mM)	Total volume	Final drug concentration
2	2	194 µL	4 µL	200 µL	20 µM
6	6 µL	592 µL	12 µL	600 µL	20 µM

If you need a low irinotecan concentration, you should prepare a low working solution at 0.1 mM or lower so that you don't have to take 0.1 uL, which is not accurate. Instead, you will take 1 2, .. uL.