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HCD Extraction Kit

Quantity: 50 reactions
Buffer Storage : 2-8 °C
Shipment: Ice gel
Lysis Mix Storage: -20 °C
Shipment: Dry Ice
Cat. No.: LG9751

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

EKB-51/1

HCD Extraction Kit

Kit Component

Component	Volume	Storage
Lysis Buffer	6 ml	2-8 °C
Extraction Buffer	6 ml	2-8 °C
Lysis Mix	300 µl	-20 °C

Shipping and Storage Condition

The HCD Extraction kit is shipped on ice gel and should be stored immediately upon receipt at 2-8°C and also, Lysis Mix is shipped separately on dry ice and should be stored immediately upon receipt at -20°C.

Equipment Required (not included)

1. 1.5 ml sterile (DNase and RNase free) microcentrifuge tubes
2. Pipettes with corresponding tips (10, 100, 1000 µl)

Preparation of Standard Samples

In order to make standard samples from the Standard mix 10 ng/µl (available in HCD Detection Kit), a 10-fold serial dilution must be prepared by taking a known volume of standard mix and placing it into a known volume of distilled water or TE buffer as explained below.

1. Label 5 microtubes as D1, D2, D3, D4, and D5. Then, take the Standard mix tube from the freezer, and after the DNA thaws, incubate it along with distilled water (or TE Buffer) at 55 °C for 15 min.
2. Vortex the standard mix shortly and spin briefly before use.
3. Add 90 µl of distilled water (or TE buffer) to microtube D1.
4. Add 180 µl of distilled water (or TE Buffer) to microtubes D2, D3, D4 and D5.
5. Take 10 µl from standard mix and add it to the microtube D1 containing 90 µl distilled water (or TE buffer) to produce a 10-fold dilution and vortex the prepared dilution vigorously before adding it to microtube D2.
6. Take 20 µl of the DNA from microtube D1 to microtube D2, then vortex severely and quick-spin.
7. Repeat step 6 several times (transfer 20 µl of DNA from the previous dilution microtube to the next one) until you add DNA to the microtube D5, to produce Standard dilutions.
8. Check the table below for the final dilutions:

Serial dilution tubes (D)	Dilution preparation	DNA/reaction (pg)
Standard Mix	Standard Mix tube (10 ng/ µl)	-
D1	10 µl Standard control + 90 µl DW*	7000 pg
D2	20 µl D1 + 180 µl DW	700 pg
D3	20 µl D2 + 180 µl DW	70 pg
D4	20 µl D3 + 180 µl DW	7 pg
D5	20 µl D4 + 180 µl DW	0.7 pg

***Distilled water (or TE buffer)**

Note: Prepare the serial dilutions on the experiment day.

Preparations of the Drug sample

1. Prepare 50 µl Drug Sample in 1.5 ml tube.
2. Add 50 µl of desired standard dilution to 50 µl Drug Sample as the spike sample.
3. Add 10 µl Lysis mix and 200 µl Lysis Buffer and mix by briefly vortex.

Then Incubate the tube at 55°C.

Drug Concentration	Recommended Incubation time
Up to 30 mg/ml	3 hrs
Over 30 mg/ml or biphasic solutions	5 hrs

4. Incubate the tube at 95°C for 15 min.
5. Add 200 µl Extraction Buffer and immediately mix thoroughly by vortex.
6. Add 1 volume cold Isopropanol (-20°C). Incubate the tube preferably at -70°C for 60 min or at -20°C overnight.
7. Centrifuge at 11000 x g for 15 min at 4°C.
8. Discard the supernatant and rinse the pellet with 1 ml of cold ethanol 70% (-20°C). Centrifuge at 11000 x g for 10 min at 4°C.
9. Discard the supernatant and dry the pellet at room temperature.
10. Dissolve the pellet in 50 µl pre-warmed (55°C) nuclease-free water or TE buffer.

11. Incubate the dissolved pellet at 55°C for 15 min or for Concentrated drugs at 95°C for 80 min. It is highly recommended to use plastic paraffin film for sealing the tubes while incubating at 95°C.
12. For optimal result, it is recommended to spin the tubes shortly after the incubation step.
13. Add 7µl of extracted DNA from drug sample, to separate Real-time PCR tube as template.
14. Add 7µl of extracted DNA from spike sample, to separate Real-time PCR tube as template.

Troubleshooting

Observation	Action
Recovery rate of spiked sample is observed out of defined range (80-120%).	- The drug could be diluted (e.g 1:2 or 1:10) Using WFI and proceed the extraction process using the diluted drug. For final amount multiplying the diluent factor in amount of DNA (pg) calculated by the software (instrument).
	- Increase incubation time (1-2 hours) for the drug and spiked sample at 55 °C.
	- For concentrated drug incubation of extracted DNA at 95 °C (30-80 min) just before qPCR assay could be helpful.