Troubleshooting

Observation	Possible Cause	Action
Unacceptable %Recovery	Pipetting error	Repeat the assay. Make sure to pipet precise amount of solution into all wells.
	Inefficient DNA extraction	Repeat DNA extraction carefully for avoiding systematic errors.
	Presence of PCR inhibitor in the eluted DNA solution	−Repeat the washing step. −Dissolve the DNA pellet completely using pre-warmed TE buffer or nuclease-free water at 55°C. (This step sometimes needs 1-2 hours to be accomplished.) −In case of concentrated drugs (>30 mg/ml), it is essential to incubate the extracted DNA at 95°C for 60-80 min before adding into real-time PCR reaction. In addition, in some cases where the buffer or matrix of examined drug interrupts with the detection assay, diluting the drug could be helpful.

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HCD (Sp2/0) ContaFind Kit
DNA Detection Kit

Quantity: 50 reactions
Store: -20 °C
Cat. No.: LG9841
Shipment: Dry ice

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

HCD (Sp2/0) ContaFind Kit
DNA Detection Kit

Kit Component

Component	Volume	Storage
ContaFind Mix (2X)	500 µl	-20°C
Probe/Primer Mix	150 µl	-20°C
Standard Mix (10 ng/µl)	100 µl	-20°C
Nuclease-free Water	500 µl	-20°C

Shipping and Storage Condition

The DNA detection kit is shipped on dry ice and should be stored immediately upon receipt at –20°C in a constant-temperature freezer.

Equipment Required (not included)

1. Real-time Apparatus
2. PCR reaction tubes for the specific real-time PCR device
3. 1.5 ml sterile (DNase and RNase free) microcentrifuge tubes
4. Pipettes with corresponding tips (10, 100, 1000 µl)

Troubleshooting

Observation	Possible Cause	Action
No positive-control or target specific signal is detected in standard/known positive tubes	Improper storage of ContaFind Mix	−Repeat the assay using properly stored assay components. −Avoid freezing and thawing assay components. Protect ContaFind Mix from light.
	Improper storage of target-specific Probe/Primer Mix	
	Pipetting error	Repeat the assay. Make sure to pipet all mixed components into all positive-control wells.
Target-specific signal is detected in negative control tubes	Carryover contamination	−Repeat the assay using fresh aliquots of all reagents and clean pipetting equipment. −If the negative control continues to show contamination, repeat the assay using a new kit. −If the negative control continues to show contamination, contact your Application Specialist.
	Inappropriate equipment/Incorrect application of materials	−Use premium quality transparent tubes specified for real-time PCR machine. −Avoid freezing and thawing assay components. Protect Master Mix from light.

12. Place the tubes in real-time PCR machine.

13. Use the following protocol for real-time PCR:

Step	Process	Temperature	Time	Cycle
1	Initial Denaturation	95°C	15 min	1
2	Denaturation	95°C	50 sec	45
	Annealing and Extension	64°C	50 sec	

Interpretation of Results

In case of DNA detection in drug, proceed to the following to find the quantity (amount of DNA (pg) in 50 µl of the drug sample):

$Y = (X \times 50)/7$

X: the amount of DNA (pg) calculated by the software (instrument).

Y: The amount of DNA (pg) per 50 µl of the drug sample.

Note: NTC/NC peak observation at Ct ≥ 35 is not valid.

Sensitivity

In order to determine the sensitivity of each Real-time PCR reaction, a dilution series must be prepared from 10 ng/µl standard mix down to 0.01 pg/µl and be used as Real-time PCR template. The LOD was 0.1 fg DNA per each Real-time PCR reaction (0.01 pg/ml) and The LOQ was 0.3 fg DNA per each Real-time PCR reaction (0.04 pg/ml).

Limitations of product use

- HCD (Sp2/0) ContaFind Kit (DNA Detection Kit) is intended for molecular biology applications in research and is not designed to apply for clinical use.
- It is important to check pipettes regularly. Cross contamination between samples and exogenous DNA can only be avoided by following good laboratory practice.
- This test has been validated for use with the reagents provided in the kit. The use of other reagents or methods, or the use of equipment not fulfilling the specifications, may result in equivocal results.
- Attention should be paid to expiration dates printed on the kit box. Do not use expired product.
- It is essential to transfer the kit to the appropriate storage condition immediately after use.

Precautions for Real-time PCR

- Thaw all components thoroughly at room temperature before starting the assay and mix the components with spin briefly. Then, add it to the reaction mix in a separate facility.
- Work quickly on ice or in the Cooling Block and always use disposable powder-free gloves.
- For serial dilution preparation, you must use Vortex mixer.

Preparation of Standard Samples

In order to make standard samples from the standard mix (10 ng/µl), 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, D5, and D6. 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, D5, and D6.
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 D6, to produce Standard dilutions.
8. Take 7 µl from standard dilution microtubes and add them to separate Real-time PCR tubes as template.
9. 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
D6 (optional)	20 µl D5 + 180 µl DW	0.07 pg

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

Note: Prepare the serial dilutions on the experiment day.

10. Select the channel for acquisition.

No	Name of channel	Source wavelength (nm)	Detection wavelength (nm)
1	FAM	470	510

11. In order to check the Sp2/0 host cell DNA contamination of 1 sample (including 1 Drug sample + 1 Spike sample (optional) + 5 Standards + 1 Negative Control) in 20 µl total volume, see the table below:

Component	Volume
ContaFind Mix (2X)	1/2 of total volume
Probe/Primer Mix	3 µl
Template	7µl
Nuclease-free Water	Up to 20 µl
Total Volume	**20 µl**

Note: In order to increase the accuracy of results, it is highly recommend performing the tests in duplicate or preferably triplicate.