DNA RapidExtract™ Kit – Troubleshooting Guide

Observation: amplification is not evident after extracting DNA from sample using the DNA RapidExtract kit.

Assumption: PCR assay is working, as evidenced by a positive control in the same experiment.

There are three possible explanations for the absence of amplification:

A. Not enough target DNA available in the current amount of starting material.
B. Amplification is inhibited by impurities, or by components of the RapidExtract kit.
C. Not enough DNA is released during the RapidExtract procedure.

Troubleshooting steps

A. Determine if there is enough DNA in the sample

1. Perform DNA extraction by a current process to use it as a positive-control extraction method. Start with the same amount of sample material as used in the RapidExtract protocol. Perform the RapidExtract protocol in parallel, on the same amount of the same material.
2. Perform amplification on the extracted material from step 1 in parallel.
3. If there are no visible PCR results after using the two DNA extraction protocols, then there is not enough DNA in the starting material. The amount of starting material should be revised.
4. If amplification is present with the positive-control extraction method, then proceed with troubleshooting the PCR inhibition.

B. Determine if PCR is inhibited

1. Serially dilute the RapidExtract-derived material with water, using the dilution factor of 5. For example, take 5 µl of the RapidExtract material and add 20 µl of water. This will constitute the 1:5 dilution. Mix well. Take 5 µl of the 1:5 dilution and add 20 µl of water. This will constitute the 1:25 dilution. Continue until you reach the 1:125 dilution. Perform the same serial dilutions for the extracted material using the positive-control extraction method.
2. In parallel, amplify undiluted and all diluted materials from two extraction protocols.

3. If the amplification is evident for one of the dilution factors, then such dilution should be incorporated into the experimental workflow. The amplification of the positive-control extraction material of the same dilution factor can serve as a control.
4. If there is no amplification with RapidExtract-derived material for any of the dilution factors, proceed with troubleshooting the efficiency of the RapidExtract process on your sample material.

C. Determine the efficiency of the RapidExtract process

1. Perform the RapidExtract procedure using sample already extracted by the positive-control method, while maintaining the original extraction volume.
2. Perform the RapidExtract procedure using the sample already extracted by the positive-control method, but now mixed with your sample material.
3. Continue with steps B-1 and B-2.
4. If the amplification is evident in one of the reactions, then the RapidExtract procedure should be optimized following suggestions in the Diffinity DNA RapidExtract™ Kit Package Insert:
    - The starting material, if solid, should be mechanically disrupted
    - The incubation time at 98 ºC may be extended up to 5 minutes

Please direct any future technical inquiries to questions@chiraltech.com