

DNA: Removal of Inhibitors Using the BioRobot® EZ1 and EZ1 Advanced XL

Principle

The presence of an inhibitor can be recognized by evaluating the internal PCR control (IPC) and threshold cycle (Ct) values in a real-time qPCR assay. The DNA concentration reported may decrease or be undetermined relative to the actual unknown amount within a sample. The analysts have the responsibility of evaluating available methods to overcome inhibition which include diluting and re-quantitating a sample, repeating the DNA extraction, or using the BioRobot® EZ1 or EZ1 Advanced XL.

DNA extracts exhibiting partial inhibition (a DNA concentration value is reported) may be purified with either BioRobot®. DNA extracts exhibiting complete inhibition (undetermined DNA concentration) must be evaluated on a sample-by-sample basis. The associated reagent blank should also be included with the inhibited DNA extracts being processed.

Materials and Equipment

This procedure uses the following materials and equipment:

- EZ1 DNA Investigator Kit
- TE Buffer
- EZ1 DNA Investigator protocol card
- BioRobot® EZ1 or EZ1 Advanced XL workstation
- vortex
- pipettes and barrier pipette tips
- microcentrifuge tubes

Pre-treatment steps

The following procedure is used to remove inhibitors prior to analysis using the BioRobot®.

Step	Action
1	To a tube of inhibited DNA extract and its accompanying reagent blank, add a sufficient volume of TE buffer to dilute the samples to no more than 200 µl.
2	Vortex the tubes briefly. Transfer the diluted samples to EZ1 sample tubes.
3	Proceed to Set-up and operation of the BioRobot EZ1 in DNA: BioRobot EZ1 and EZ1 Advanced XL Extraction.