

5 Critical Reagents

Extraction Reagents

Each lot of a DNA extraction reagent or a DNA extraction kit must be quality control tested prior to use on casework to establish both functionality and a lack of contamination. Digestion components not received with or included in a kit but used in conjunction with a kit, such as QIAGEN MTL buffer or additional QIAGEN proteinase K, must also be quality control tested. At least one appropriate sample of known origin and one reagent blank must be incorporated into the quality control test. A sample is considered appropriate if it is similar to samples generally encountered through the use of a particular reagent (i.e, when testing DTT, a semen-containing sample must be used). All documentation generated through the quality control testing should accompany the reagent quality control form (e.g, extraction, quantification, and amplification worksheets, electropherograms, etc.) and be maintained in the laboratory. It is acceptable to quality control test multiple reagents simultaneously, but unacceptable results may require individual retesting to troubleshoot appropriately. Acceptable results include results for the known sample that are concordant with previously obtained data and a reagent blank free of contaminating DNA (no activity that cannot readily be attributed to an amplification or electrophoretic artifact). Reagent blank samples must be subjected to the most sensitive volume and injection conditions possible. Questionable results must be brought to the attention of the Technical Leader immediately.

The following extraction reagents are considered critical reagents and must be quality control tested prior to use on casework samples:

- Sterile di water
- Digest buffer
- DTT, 0.39M
- DTT, 1M
- Ethanol, denatured
- EZ1 DNA Investigator Kit (QIAGEN)
 - Buffer G2
 - Proteinase K
 - Carrier RNA
- MTL Buffer
- Phenol:Chloroform:Isoamyl alcohol (v/v 25:24:1)
- Proteinase K, 10 mg/mL
- QIAamp® DNA Mini Kit
 - Buffer AL
 - Buffer ATL
 - Buffer AW1 concentrate
 - Buffer AW2 concentrate
 - Buffer AE
 - Proteinase K (20 mg/ml, 600 mAU/ml solution or 40 mAU/mg protein)
- Sarcosyl, 20%
- Stain Extraction Buffer
- TE Buffer
- TNE Buffer

Quantification Kits

Each lot of a DNA quantification kit must be quality control tested prior to use on casework to establish both functionality and a lack of contamination. All documentation generated through the quality control testing should accompany the reagent quality control form (e.g., quantification worksheets) and be maintained in the laboratory. Questionable results must be brought to the attention of the Technical Leader immediately.

Acceptable results include:

1. The control DNA must be diluted to make a standard curve according to the procedure outlined in the sectional SOP. This DNA must then be run in duplicate on a plate. IPC values must be obtained from all points on the curve to ensure the reagents are working correctly and the standard curve must be within acceptable ranges for slope, y-intercept, and r^2 values, without having to remove more than 3 points.
2. A negative control consisting of master mix and TE buffer or Dilution Buffer (whichever reagent was used to create the standards) must be run on the same plate. This negative control must have an IPC value and less than 5×10^{-3} ng/ μ l of detectable DNA present.

Amplification Kits

Each lot of a DNA amplification kit must be quality control tested prior to use on casework to establish both functionality and a lack of contamination. All documentation generated through the quality control testing should accompany this form (e.g, amplification worksheets, electropherograms, etc.). Negative samples must be subjected to the most sensitive volume and injection conditions possible. Questionable results must be brought to the attention of the Technical Leader immediately.

Acceptable results include:

1. The positive control DNA must be run and the correct profile (as reported by the manufacturer) must be obtained.
2. An amplification blank must be run and shown to have no detectable alleles (no activity that cannot readily be attributed to an amplification or electrophoretic artifact).
3. All reagents in the kit must be used.
4. The allelic ladder must be run to determine that all of the appropriate alleles are detected.