



REFERENCE MANUAL

Validation Guide for the DNA IQ™ Casework Pro Kit for Maxwell® 16

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1. Introduction

Before implementing an automated DNA extraction system (a combination of robot and extraction chemistry) for use with casework samples, each laboratory needs to complete an internal validation study of the automated system. Validation studies are intended to show that the automated system yields DNA templates with subsequent STR genotypes that are concordant with current manual extraction procedures used in the laboratory and have no detectable levels of cross-contamination. In addition this validation study will demonstrate the yields obtained with the DNA IQ™ System for various casework sample types routinely extracted in the laboratory.

This document provides a guide to suggested experiments for the internal validation of the DNA IQ™ Casework Pro Kit for Maxwell® 16^(a). This documentation is meant to provide general guidance and should be considered along with your laboratory's standard validation protocols and accreditation requirements. A suggested minimum number of samples that need to be processed is also provided. Laboratories should determine the appropriate number and types of samples that need to be processed based on their current laboratory guidelines and/or protocols.

Please refer to the *DNA IQ™ Casework Pro Kit for Maxwell® 16 Technical Manual #TM332* for specific protocol and troubleshooting information for the DNA IQ™ Casework Pro Kit.

2. Reproducibility of Known and Non-Probativ e Samples

The objective of the reproducibility study is to demonstrate that there is no difference (discordance) between the STR profile resulting from amplification of DNA purified using the DNA IQ™ Casework Pro Kit for Maxwell® 16 and the STR profile obtained following amplification of DNA extracted using the laboratory's current procedures.

Testing Conditions

1. Using the DNA IQ™ Casework Pro Kit for Maxwell® 16, extract DNA from at least 10 samples (known, nonprobativ e, differential extraction or any combination) for which the STR profile has been previously determined using your laboratory's current extraction procedure. It is suggested that these samples include a variety of the sample types and pre-processing protocols the laboratory expects to use.
2. Quantify the samples, and amplify the mass of DNA template as defined by your laboratory protocol.
3. Determine the resulting STR profile using the laboratory's standard analysis parameters and macros (for laboratories using GeneScan® and Genotyper® software) or panel and bin files (for laboratories using GeneMapper® *ID* software), and verify that the correct STR profile was obtained.

3. Cross-Contamination

The objective of the cross-contamination study is to demonstrate that there is no detectable cross-contamination between adjacent extractions performed using the DNA IQ™ Casework Pro Kit for Maxwell® 16. This study may be performed in conjunction with any of the other studies described in this document.

Testing Conditions

1. Extract 8 samples containing DNA and 8 blanks in an alternating pattern across the tray of DNA IQ™ Casework Pro Kit for Maxwell® 16 cartridges.
2. Repeat twice for a total of three runs to demonstrate consistency.
3. Quantify the samples, and amplify the same volume or mass of DNA template as required following your laboratory's protocol for negative control or reagent blank samples.
4. Analyze the resulting electropherograms using your laboratory's standard analysis parameters for negative-control or reagent-blank samples.
5. Compare any profiles obtained from blanks to those obtained from samples to check for potential cross-contamination during extraction, as opposed to contamination introduced post-extraction.

4. DNA Yield

The objective of the yield study is to demonstrate the mass of DNA that can be obtained from a range of sample types and volumes, including limiting samples.

1. Extract 3 different volumes each of human blood and semen in triplicate (1 μ l, 0.1 μ l and 0.01 μ l) using the DNA IQ™ Casework Pro Kit for Maxwell® 16. The samples can be prepared by creating 1:50, 1:500 and 1:5000 dilutions of each of the liquid samples in 1X PBS or similar dilution medium to prepare a stock solution. Spot 50 μ l of each diluted stock solution onto swabs or other suitable substrate. Allow the samples to dry overnight prior to extraction.
2. Quantify the samples, and amplify the same mass of DNA template as defined by your laboratory protocol.
3. Analyze the resulting electropherograms using your laboratory's standard analysis parameters to determine the STR profile resulting from the extractions. This information will aid the laboratory in identifying the limits of the DNA IQ™ Casework Pro Kit for Maxwell® 16 with limited samples. A comparison to the laboratory's current extraction protocols may be helpful.

Note: It may be helpful to count the number of white blood cells in blood and sperm cells in semen to estimate the total amount of DNA in the samples.

5. Total Number of Samples

We suggest the following minimum number of samples per test:

Reproducibility	10
Cross-Contamination	48
DNA Yield	18

This is a total of 76 samples if all the experiments are performed separately. However, the number of samples can be reduced to 52 using the reproducibility and DNA yield samples for the nonblank samples in the cross-contamination experiments. Factoring in subsequent quantitation, amplification and analysis, it is feasible to complete the entire validation in a 5-day week.

6. Optional Experiments

6.A. Mixture Study

The objective of the mixture study is to determine the lowest mixture ratio where a complete profile for both samples is still observed.

Mix two liquid blood samples with known profiles, one male (A) and one female (B), as follows, creating sufficient stock for extracting the samples in triplicate to control for sample-to-sample variation:

Mixture Ratio	Sample A	Sample B
19:1	19µl	1µl
9:1	18µl	2µl
6:1	18µl	3µl
3:1	15µl	5µl
1:1	10µl	10µl
1:3	5µl	15µl
1:6	3µl	18µl
1:9	2µl	18µl
1:19	1µl	19µl

1. Deposit 20µl of the stock solution onto each of 3 swabs for each mixture, allow them to dry overnight and then extract the full swabs using the DNA IQ™ Casework Pro Kit for Maxwell® 16.
2. Quantify the samples, and amplify the same mass of DNA template as required for your laboratory's choice of STR multiplex kit and reaction volume.
3. Analyze the electropherograms using your laboratory's standard cutoff threshold (e.g., 100 or 150RFU), and determine the STR profiles obtained. Use this data to identify the DNA dilutions at which allele and locus dropout are observed and when it is possible to obtain a complete STR profile. It may be useful to perform this experiment using the laboratory's current methods in order to compare the results to those obtained using the DNA IQ™ Casework Pro Kit for Maxwell® 16.

6.B. Substrates and Challenging Samples

The objective of the substrate study is to process several different substrate types that reflect those commonly submitted for forensic DNA analysis, including inhibited or otherwise challenging samples. The following is a list of possible sample types: hair, tissue, grass, rusty metal, dirty carpet, leather, blue denim, black denim, oily rags and samples previously processed for fingerprint analysis.

1. Deposit saliva, blood, or other sample onto any substrate you wish to test, and extract the samples using the DNA IQ™ Casework Pro Kit for Maxwell® 16.
Note: It may be helpful to count the number of white blood cells in blood and sperm cells in semen to estimate the total amount of DNA in the samples.
2. Quantify the samples, and amplify the same mass of DNA template as defined by your laboratory protocols.

- Analyze the resulting electropherograms using the laboratory's analysis parameters, and determine the STR profiles obtained. Use this data to identify sample types and substrates extracted using the DNA IQ™ Casework Pro Kit for Maxwell® 16 that yielded comparable or better results than other laboratory procedures. It may be useful to perform this experiment using the laboratory's current methods in order to compare the results to those obtained using the DNA IQ™ Casework Pro Kit for Maxwell® 16.

7. References

- Greenspoon, S. A. *et al.* (2004) Application of the BioMek 2000 Laboratory Automation Workstation and the DNA IQ System to the extraction of forensic casework samples. *J Forensic Sci.* **49(1)**, 29–39.
- Validation Guide for the DNA IQ™ Reference Sample Kit for Maxwell™ 16*, #GE181 (2006) Promega Corporation.
- Bessetti, J. (2007) An introduction to PCR inhibitors. *Profiles in DNA* **10(1)**, 9–10.

8. Related Products

Product	Size	Cat.#
Maxwell® 16 Forensic Instrument LEV*	1 each	AS3060-LC
Maxwell® 16 Forensic Instrument SEV*	1 each	AS3060-SC
DNA IQ™ Casework Pro Kit for Maxwell® 16*	48 preps	AS1240
Tissue and Hair Extraction Kit (for use with DNA IQ™)	100 reactions	DC6740
Differex™ System*	200 samples	DC6800
	50 samples	DC6801

*Not For Medical Diagnostic Use.

(a)U.S. Pat. Nos. 6,027,945, 6,368,800 and 6,673,631, Australian Pat. No. 732756, European Pat. Nos. 1 204 741, 0 895 546 and 1 367 137, Japanese Pat. No. 4425513 and other patents pending.

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