DNA
Procedures for Reagent Purchasing, Preparation, and Records

1 Scope

This document applies to DNA personnel responsible for the purchasing and receipt, preparation, and labeling of laboratory reagents and consumables used for the serological and/or DNA analysis of forensic evidence and/or databasing samples in the DNA Casework Unit (DCU), the DNA Support Unit (DSU), the Federal DNA Database Unit (FDDU), and the Scientific and Biometric Analysis Unit (SBAU).

2 Responsibilities

DNA personnel will:

- Know the location in the laboratory of the Safety Data Sheets (SDSs)
- Be aware of the health and safety hazards for the chemicals used.
- Ensure that all laboratory reagents are prepared correctly and properly labeled.
- Properly store reagents in a manner to minimize degradation.
- Inspect the reagents to ensure that they have not become visually contaminated or degraded.
- Not use deteriorated or outdated reagents and solutions.

3 Procedures

The DNA units comply with the FBI Laboratory *Quality Assurance Manual* (QAM) and *Laboratory Operations Manual* (LOM) and the *Quality Assurance Standards* (QAS) *for Forensic DNA Testing Laboratories* and *for DNA Databasing Laboratories* with regard to the quality control (QC) of reagents. Reagent records will be maintained, generally via the DNA units' Sample Tracking and Control Software (STACS).

3.1 Commercial Reagent Records

3.1.1 Purchase requests will be prepared by appropriate personnel for all commercial reagents and consumables. These requests will describe the types of supplies and/or services requested and may be maintained in a written or electronic format. The appropriate Unit Chief (UC) will approve purchase requests prior to ordering.

3.1.2 Various companies may supply one or more chemicals, reagents, or DNA analysis kits used in the analysis of forensic evidence and/or database samples. Final selection of

suppliers will be in accordance with Federal Procurement Regulations - Simplified Acquisition Procedures.

3.1.2.1 Suppliers of critical reagents are typically evaluated during validation but the evaluation may be based on previous purchasing history and/or the results of QC testing.

3.1.2.2 When a vendor, reagent specification (e.g., concentration), or consumable specification (e.g., Vivicon filter molecular weight cut-off) affects the laboratory activities, the pertinent information will be listed in the appropriate DNA procedure.

3.1.2.3 Current supplier and purchasing information for reagents and consumables is generally maintained in STACS.

3.1.3 DNA personnel will ensure that quality affecting supplies, reagents, and consumables comply with specifications defined in the appropriate technical procedure and/or the purchase request. Any discrepancies will be brought to the attention of the personnel responsible for ordering DNA supplies, reagents, and consumables. Quality affecting supplies, reagents, and consumables that conform to the expected specifications will be received into STACS.

3.1.4 The following information will be recorded for the receipt of commercial reagents and kits:

- Name of material
- Manufacturer lot number
- Date received
- Expiration date, when provided

3.1.5 Commercial reagents must be labeled with the identity of the reagent and will be directly labeled and/or use a barcode system to electronically track the expiration date.

3.1.5.1 Commercial reagents are generally prepared and stored as recommended by the manufacturer. Additional guidance may be found in STACS or the appropriate DNA SOP.

3.1.5.2 The expiration date of commercial reagents is determined by the manufacturer or utilizing the respective SDS.

3.1.5.2.1 If no expiration date is provided by the manufacturer, generally a 10 year expiration date will be assigned.

3.1.5.2.2 If the expiration date provided by the manufacturer only specifies a month and year, the recorded expiration date will be the last day of the month specified. The commercial reagent container will then be labeled with the newly established expiration date.

3.1.5.2.3 If an expiration date beyond that provided by the manufacturer is assigned, records to establish the extension of the expiration date will be maintained.

3.1.5.2.4 If an expiration date is exceeded, the QC procedure or use of the reagent on a known sample may be used to demonstrate continued efficacy beyond the assigned expiration date. Records demonstrating the continued efficacy of the reagent will be maintained.

3.1.6 If a non-reagent consumable (e.g., swabs, tubes, microcons, centristrips) has an expiration date assigned by the manufacturer, that expiration date is not applicable to DNA units' operations and may be extended as deemed appropriate by the Technical Leader (TL). The new expiration date will be recorded in STACS.

3.2 Laboratory Prepared Reagent Records

3.2.1 All laboratory prepared reagents will be prepared in accordance with the information contained within STACS and/or the relevant DNA procedure. A list of reagent recipes and control preparation guidelines generally used in the DNA units are listed in Appendix A for reference.

3.2.2 Reagent preparation will be recorded, generally within STACS, to include:

- Name of reagent
- Date prepared
- Lot number (e.g., barcode, batch identifier) assigned to the reagent
- Lot number (e.g., barcode, batch identifier) of each component
- Initials of the preparer
- Initials of the individual performing the QC check (if applicable)
- Lot/batch # of the QC controls (if applicable)
- QC results (pass/fail) (if applicable)

3.2.2.1 The information recorded for reagent preparation and use must be sufficient to provide a documented audit trail.

3.2.2.2 Unless otherwise specified, reagents that are made internally will expire one year from the date prepared. If an expiration date is extended, records demonstrating the continued efficacy of the reagent will be maintained.

3.2.2.2.1 If an expiration date is exceeded, the use of the reagent on a known sample may be used to demonstrate continued efficacy beyond the assigned expiration date. Records demonstrating the continued efficacy of the reagent will be maintained.

3.2.3 Laboratory prepared reagents will be labeled with the identity of the reagent and will be directly labeled and/or use a barcode system to electronically track the following:

- Preparer's initials
- Date prepared
- Expiration date
- Lot number (e.g., barcode, batch identifier)

3.2.3.1 Reagents stored in limited use quantities (i.e., single use aliquots) may be in a storage container (e.g., bag, box) labeled with the identity of the reagent with the associated barcode(s) readily available.

3.2.3.2 Once dispensed for use (e.g., on a robotic workstation, on another instrument), the trough or tube will be labeled with the identity of the contained reagent with the necessary barcode available for scanning.

3.2.4 Laboratory prepared reagents will be stored at an appropriate temperature to prevent degradation or deactivation of the active ingredients. Recommended storage conditions may be listed in STACS and/or Appendix A.

3.2.5 Laboratory prepared reagents will be tested for reliability prior to or concurrent with use in casework examinations or DNA databasing. QC procedures for reliability testing may be contained in the appropriate DNA procedure or in STACS or reliability testing may be accomplished by testing appropriate positive and/or negative controls. Dilutions of a stock commercial reagent (e.g., 1X CE buffer) will not require reliability testing. Multiple reagents may be simultaneously tested for reliability.

3.3 Critical Reagents

The QAS defines critical reagents as reagents whose performance is vital to the success of the DNA testing and require testing on known samples before use on forensic or database samples. The reliability of the following critical reagents is evaluated prior to their use on evidence or databasing samples.

3.3.1 The following are identified as QAS critical reagents:

- Nuclear DNA Quantification Kits (i.e., Quantifiler DUO, Quantifiler TRIO)
- Mitochondrial DNA Quantitative PCR (qPCR) system:
  - Double stranded synthetic standard (dsT8sig)
  - TaqMan® Fast Advanced Master Mix
  - Amplification primers (Qfor8, Qrev8, L, M, G, B)
  - Probes (QRL8 [FAM], C [VIC], and U [NED])
  - Double stranded Internal Positive Control DNA (C/E)
- STR Amplification Kits (i.e., Globalfiler)
- Y-STR Amplification Kits (i.e.,Yfiler)
- Mitochondrial DNA amplification and sequencing systems:
  - 10X PCR Buffer
  - BSA
  - Amplitaq Gold
  - Primers for mtDNA amplification and sequencing
  - Deoxyribonucleotide triphosphate mix (dNTPs)
  - Big Dye Sequencing Kits
  - HL60 DNA
- EXOSap-IT

- Amplification and Sequencing components (if not within a test kit or system):
  - Amplitaq Gold
  - Primers
  - Allelic Ladders
- Rapid DNA Cartridge

3.3.2 The QC procedures used to ensure the reliability of critical reagents are contained in the appropriate DNA procedure or in STACS.

3.3.3 The results of the QC testing, as well as the reagent's acceptance or rejection for use, will be recorded. The reagent will be available for use once the necessary acceptance is recorded, generally in STACS.

4 Safety

4.1 Refer to the "Safe Work Practices and Procedures," "Bloodborne Pathogen (BBP) Exposure Control Plan (ECP)," "Personal Protective Equipment Policy," and "Chemical Hygiene Plan" sections of the *FBI Laboratory Safety Manual* for important personal safety information prior to conducting laboratory procedures.

4.2 Refer to the "Hazardous Waste Disposal" section of the *FBI Laboratory Safety Manual* for important information concerning proper disposal of the chemicals used in laboratory procedures as well as the biohazardous waste generated.

4.3 Appropriate safety precautions and proper personal protective equipment will be used during reagent preparation and performance of laboratory procedures. Refer to Safety Data Sheets, *FBI Laboratory Safety Manual*, and relevant DNA procedures for more detailed information.

5 Records

Records pertaining to the receipt, preparation, and/or QC of laboratory reagents will be kept in the STACS or an equivalent log or storage location. These records will be maintained by the DSU QA Group and/or in the applicable DNA Unit. Records verifying the completeness of each commercial order will be maintained by appropriate personnel.

6 References

FBI Laboratory Quality Assurance Manual

FBI Laboratory Operations Manual

FBI Laboratory Safety Manual

DNA Procedures Manual

Federal Bureau of Investigation, Quality Assurance Standards for DNA Databasing Laboratories, latest revision.

Federal Bureau of Investigation, Quality Assurance Standards for Forensic DNA Testing Laboratories, latest revision.

U.S Government Printing Office. Title 48 Code of Federal Regulations Part 13 (48CFR13), Federal Procurement Regulations - Simplified Acquisition Procedures.

Rev. #	Issue Date	History
8	02/15/19	Revised critical reagent list to add new mtDNA qPCR reagents and update kit examples. Removed autoclaving instructions from Appendix A. Adjusted reagent recipes to reflect current default volumes. Added new qPCR reagent recipes. Added SEB+DTT recipe.
9	02/18/20	Updated to BAU to SBAU Added consumables as appropriate 3.1.2.1 Reworded as most evaluations are done through validation 3.1.2.2 Added example of consumable specification 3.1.2.3 Removed separate listing since STACS is generally used to maintain suppliers for reagents and consumables 3.2.5 Added testing appropriate controls 3.3 Updated QAS definition. Changed tested to evaluated 3.3.1 Added Rapid DNA cartridges App A Added Bode Collector as an option for making BIS

Approval Redacted - Signatures on File

DNA Technical Leader Date: 02/14/2020

DCU Chief Date: 02/14/2020

DSU Chief Date: 02/14/2020

FDDU Chief Date: 02/14/2020

SBAU Chief Date: 02/14/2020

QA Approval

Quality Manager Date: 02/14/2020

Appendix A: *Reagent Recipes and Control Preparation*

Reagent Preparation Guidance:

If more or less reagent is needed than what is listed below, the components should be adjusted proportionally to make the volume needed. Graduated cylinders and/or pipettes closest in capacity to the volume of liquid being measured should be used. If the pH meter is used, the performance will be verified prior to use. Any reagent in which microbial growth is observed must be discarded. Store all reagents in sterile containers unless otherwise noted. When available, purchased ready to use reagents of equivalent or higher quality may be substituted for the reagents listed below.

1X Genetic Analyzer Buffer with EDTA

- Combine 100 mL of 10X genetic analyzer buffer with EDTA with 900 mL reagent grade water.
  - For single reservoir on a 3130XL: Combine 3.5 ml 10X Genetic Analyzer buffer with EDTA with 31.5 mL reagent grade water
  - For single set-up on a 3730: Combine 20 mL of 10X Genetic Analyzer buffer with EDTA with 180 mL of reagent grade water
- Store refrigerated for up to 1 month.

Acid Phosphatase Spot Test Solution, 100 mL

- Add 2.6 g Acid Phosphatase Spot Test powder to 100 mL reagent grade water and stir until dissolved.
- Store frozen for up to one month.

3% Bleach Solution, 100 mL (For TECAN only)

- Dilute 3 mL molecular grade bleach (or equivalent) to 100 mL with reagent grade water.
- Store at room temperature.
- Prepare daily.

10% Bleach Solution, 50 mL (for use on evidentiary items)

- Dilute 5 mL household bleach (or equivalent) to 50 mL with reagent grade water.
- Store at room temperature.
- Prepare daily.

10% Bleach Solution, 10 L (for cleaning purposes)

- Dilute 1 L household bleach (or equivalent) to 10 L with reverse osmosis

(RO) purified water.
- Store at room temperature.
- Prepare at least weekly.

Bovine Serum Albumin (BSA), 1.6 mg/mL, 80 mL

- Add less than 80 mL of reagent grade water to a container.
- Add 128 mg of bovine serum albumin.
- Bring final volume to 80 mL with reagent grade water.
- Store frozen.

Demineralization/Extraction Buffer, 1L

- Add 900 mL of 0.5M EDTA solution to a container and stir on medium.
- While stirring, add 10g of N-Lauroylsarcosine sodium salt.
- Allow mixture to go into solution.
- Adjust to pH 8.0 with hydrochloric acid (HCl) or Sodium Hydroxide (NaOH).
- Bring final volume to 1 L with 0.5M EDTA solution.
- Store at room temperature.

1M DTT (Dithiothreitol), 10 mL

- Dissolve 1.54 g of DTT in 10 mL of reagent grade water.
- Store frozen.

5M DTT (Dithiothreitol), 2 mL

- Dissolve 1.54 g of DTT in 2 mL of reagent grade water.
- Store frozen.

70% Ethyl Alcohol (EtOH), 100 mL

- Dilute 74 mL 95% ethyl alcohol to 100 mL with reagent grade water.
- Store at room temperature.

HEPES-Buffered Saline (HBS), 1 L (10mM HEPES / 144 mM NaCl / pH 7.2)

- Dissolve 8.42 g NaCl in 900 mL reagent grade water.
- Add 2.38 g HEPES and stir until dissolved.
- Adjust to pH 7.2 with 2.5 M NaOH.
- Bring final volume to 1 L with reagent grade water.
- Store refrigerated.

3% Hydrogen Peroxide Solution, 1 L

- Dilute 100 mL of 30% hydrogen peroxide solution to 1 L with reagent grade water.
- Store refrigerated.

mtDNA Amplification Primers

Amplification primers will be initially hydrated with TE^{-4} to a stock concentration of 100 µM. The stock concentration will be diluted to a working concentration of 30 µM (A1, A2, B1, B2, C1, C2, D1, D2, 617) or 10 µM (miniprimers) as appropriate.

Calculation:

Optical Density (OD) (A260) / Extinction Coefficient (OD units/µmole) = µmole of primer
100 µM = 100 µmole/L = 100 µmole/1000 mL = 0.1 µmole/mL
= 0.1 µmole/1000 µl = 0.0001 µmole/µl
µmole of primer / 0.0001 µmole/µl = µl TE^{-4} for 100 µM solution

(The OD and Extinction Coefficient can be found on the certificate for each primer.)

Prepare a 100 µM stock solution from lyophilized primer

- Add 1 to 2 mL of TE^{-4} to vendor tube with lyophilized primer (depending on tube size) and vortex.
- Let sit for ~5 min at room temperature and vortex again.
- Transfer liquid from vendor tube to a 50 mL conical tube.
- Add an additional 1 to 2 mL of TE^{-4} to vendor tube, vortex, and transfer to same 50 mL conical tube.
- Add remaining amount of TE^{-4} to bring solution to volume determined by calculation. (Remember to subtract the initial 2-4 mL used)
- This will be the 100 µM stock primer solution (vortex before transferring to other tubes) used to prepare the 30 µM, 10 µM and 1 µM primer solutions as needed.

Prepare a 30 µM solution for primers A1, A2, B1, B2, C1, C2, D1, D2, 617 from the 100 µM stock solution and store frozen.

A1 primer:

- Transfer 4.5mL of 100 µM stock solution to a new 50 mL conical tube.
- Add 10.5 mL of TE^{-4}.

All other primers:

- Transfer 3.15 mL of 100 µM stock solution to a new 50 mL conical tube.

- Add 7.35 mL of TE^{-4}.

Prepare a 10 µM primer solution for all miniprimers from the 100 µM stock solution.

- Transfer 300 µl of 100 µM stock solution to a new 50 mL conical tube.
- Add 2700 µL of TE^{-4}.

mtDNA Quantitative PCR IPC - Double Stranded Internal Positive Control DNA (C/E)

- Reconstitute forward (C) and reverse (E) oligonucleotides in TE^{-4} buffer.
- Prepare 100 µM solutions of the forward and reverse oligonucleotides using information from vendor certificate of analysis.
- Combine these in equal volumes and tightly cap, mix, and quick spin the tube.
- This generates the **primary (1°) stock** of the double-stranded IPC DNA at 50 µM (3×10^{13} copies/µl). Store frozen.
- Prepare a dilution series using TE^{-4} as follows:
  - 2º stock: Transfer 10 µl of primary stock into 1,542 µl TE^{-4} (1.94×10^{11} copies/µl).
  - 3º stock: Transfer 10 µl of 2º stock into 1,542 µl TE^{-4} (1.25×10^{9} copies/µl).
  - 4º stock: Transfer 10 µl of 3º stock into 990 µl TE^{-4} (1.25×10^{7} copies/µl).
  - 5º stock: Transfer 10 µl of 4º stock into 990 µl TE^{-4} (1.25×10^{5} copies/µl).
  - 6º stock (working dilution): Transfer 10 µl of 5º stock into 990 µl TE^{-4} (1.25×10^{3} copies/µl).
- Store frozen.

mtDNA Quantitative PCR Primers (Forward [Qfor8, L, G] and Reverse [Qrev8, M, B])

- Reconstitute all primers in TE^{-4} buffer.
- Prepare 100 µM stock solutions of each primer using information from vendor certificate of analysis.
- Store frozen.
- Prepare working dilutions of each primer in TE^{-4} buffer as follows:
  - For Qfor8 and G (1.25 µM) transfer 12.5 µl of 100 µM stock solution to a new tube and add 987.5 µL of TE^{-4}.
  - For Qrev8 and M (22.5 µM) transfer 225 µl of 100 µM stock solution to a new tube and add 775 µL of TE^{-4}.
  - For L and B (7.5 µM) transfer 75 µl of 100 µM stock solution to a new tube and add 925 µL of TE^{-4}.
- Store frozen.

mtDNA Quantitative PCR Probes (QRL8 [FAM], C [VIC], U [NED])

- Prepare a 6.25 µM working dilution from each 100 µM probe stock.
  - Transfer 62.5 µl of 100 µM stock solution to a new tube and add 937.5 µl of TE^{-4}.

- Store frozen and protected from light as much as possible.

mtDNA Quantitative PCR Primer/Probe/IPC Mix (PPI Mix)

- Prepare working dilutions of all primers, probes, and IPC DNA.
- Add 80 µL of all primers, probes, and IPC DNA into each tube. Vortex, pulse spin.
- Store frozen.

mtDNA Quantitative PCR Standard - Double Stranded Synthetic Standard (dsT8sig)

- Reconstitute Tfor8sig and Trev8sig oligonucleotides in TE^{-4} buffer.
- Prepare 2 µM solutions of the forward and reverse oligonucleotides based on their respective molecular weights of 34,960.7 g/mol and 35,969.3 g/mol.
- Combine these in equal volumes and tightly cap, mix, and quick spin the tube.
- This generates the **primary (1°) stock** of the double-stranded **dsT8sig** standard at 1 µM (6.023×10^{11} copies/µL). Store frozen.
- Prepare the **secondary (2°) stock** of **dsT8sig** from the **1° stock** using TE^{-4} buffer. The **2° stock of dsT8sig** should be at a final concentration of 5×10^{9} copies/µL.
- Add 1194.6 µL TE^{-4} buffer to 10 µL aliquot of **primary (1°) stock.** Tightly cap, mix, and quick spin the tube.
- Store frozen.

mtDNA Sequencing Primers (1 µM)

New lots of primers used in cycle sequencing will be diluted from the 100 µM primer stocks.

Prepare a 1 µM solution from the 100 µM stock solution and store frozen:

A1 primer:

- Transfer 240 µl of 100 µM stock solution to a new 50 mL conical tube.
- Add 23,760 µL of TE^{-4}.

All other primers:

- Transfer 150 µl of 100 µM stock solution to a new 50 mL conical tube.
- Add 14,850 µL of TE^{-4}.

Miniprimers:

- Transfer 50 µl of 100 µM stock solution to a new 50 mL conical tube.
- Add 4950 µL of TE^{-4}.

Phenolphthalin Solution, 1 L

- Combine 4 g phenolphthalin, 40 g NaOH, and 200 mL reagent grade water.
- Add 800 mL of ethanol and mix.
- Store refrigerated in an amber bottle over zinc (generally enough to cover the bottom of the bottle).
- This solution may be used for up to 3 months.

Saturated D-Glucose Solution

- Dissolve 10 g of dextrose in 10 mL reagent grade water with mild heating.
- Store at room temperature for up to 1 month.

2.5 M (10% w/v) Sodium Hydroxide (NaOH), 100 mL

- Add 10 g of NaOH to 80 mL reagent grade water.
- Store at room temperature.

Sperm Wash Buffer, 1 L (10 mM Tris-HCl / 10 mM EDTA / 50 mM NaCl / 2% SDS, pH 8.0)

- Add 10 mL 1 M Tris-HCl, 20 mL 0.5 M EDTA, 10 mL 5 M NaCl, and 100 mL 20% SDS to approximately 800 mL reagent grade water.
- Adjust to pH 8.0 with HCl or NaOH.
- Adjust the final volume to 1 L with reagent grade water.
- Store at room temperature.

Stain Extraction Buffer (SEB), 1 L (10 mM Tris-HCl / 100 mM NaCl / 10 mM EDTA / 2% SDS, pH 8.0)

- Dissolve 5.84 g NaCl in approximately 500 ml reagent grade water.
- Add 10 mL 1M Tris-HCl.
- 20 ml 0.5M EDTA.
- Add 100 mL 20% SDS.
- Adjust to pH 8.0 with HCl.
- Adjust the final volume to 1 L with reagent grade water.
- Store at room temperature.

SEB with Dithiothrietol (SEB w/DTT), 5 mL (10 mM Tris-HCl / 100 mM NaCl / 10 mM EDTA / 2% SDS / 39mM DTT)

- Add 30 mg of DTT to 5 mL of SEB and stir until dissolved.
- Store at room temperature for up to 1 month.

Takayama Hemochromogen Reagent

In a chemical fume hood, combine:

- Add 1 mL saturated D-glucose solution
- Add 1 mL 2.5 M NaOH
- Add 1 mL pyridine with 2 mL reagent grade water.
- Mix thoroughly.
- Store in an amber bottle at room temperature for up to 1 week.

TNE, 1 L (10 mM Tris-HCl / 100 mM NaCl / 1 mM EDTA, pH 8.0)

- Add 10 mL 1 M Tris-HCl
- Add 5.84 g of NaCl
- Add 2 mL 0.5 M EDTA to 750 mL reagent grade water.
- Adjust to pH 8.0 with HCl or NaOH as necessary.
- Adjust the final volume to 1 L with reagent grade water.
- Store refrigerated.

Control Preparation Guidance:

Blood/Buccal Internal Standard (BIS)

An individual providing the BIS control must have a previously characterized and documented STR profile in the FDDU.

- A blood BIS control is an FTA bloodstain card prepared with liquid blood or blood collected via a finger stick.
- A buccal BIS control is the FTA card from a Whatman EasiCollect™ device which is used to collect and transfer a buccal sample to the card, the collector from a Bode Collector device used for a buccal collection, or an equivalent card collected using an approved collection device.

Blood Known Positive Swab

- Prepare a 1:250 dilution of whole blood.
- Add approximately 100 µL of diluted blood to each clean swab.
- Allow the swabs to dry completely.
- Store refrigerated or at room temperature.

mtDNA NIST Standard Reference Material - NIST SRM 2392-I (HL60)

Prepare a 140 pg/µl dilution from 1.4 ng/uL stock:

- Transfer 10 µL of stock to a new 1.5 mL tube.
- Add 90 µL of TE^{-4}.

mtDNA Positive Control (HL60)

Calculation:

Concentration of HL60 (stock) x V1 = 10 ng/µL x 200 µL
V1 = amount of stock needed to make a 10 ng/µL solution
200 µL – V1 = amount of TE^{-4} needed to make a 10 ng/µL solution

Prepare a 10 ng/µL stock solution from the vendor stock of the HL60 DNA.

- Add amount of stock and TE^{-4} determined by above calculation.
- Store frozen.

Prepare a 20 pg/µL solution from the 10 ng/µL stock.

- Add 19,960 µL of TE^{-4} to 40 µL of the 10 ng/µL solution.
- Store frozen.

Prepare a 100 pg/µL solution from the 10 ng/µL stock.

- Add 14,850 µl of TE^{-4} to 150 µL of the 10 ng/µL solution.
- Store frozen.

Semen Known Positive Slide

- Prepare a 1:10 dilution of neat human semen with reagent grade water.
- Pipette 4µL of diluted semen to center of microscope slide.
- Allow slide to air dry (generally for 10 minutes).
- Store at room temperature.

Semen Known Positive Swab Preparation

- Add 1.5 mL of human semen to 3.0 mL of reagent grade water.
- Add approximately 100 µL of diluted semen to each swab.
- Allow the swabs to dry completely.
- Store frozen.