**PCR Interfering Substances: BSA Method Procedure**

1. **PRINCIPLE:**
   1. Interfering substances present in DNA/RNA are known to interfere with PCR amplification.
      1. This can include excess salts, ionic detergents, SDS, ethanol, isopropanol, phenol and other substances used in the process of DNA extraction.
      2. In addition, any substance that interferes with Mg++ can inhibit PCR.
      3. Following standard protocol and good technique can eliminate this type of inhibition in most cases.
   2. However, hemoglobin and melanin are also known to inhibit PCR amplification.
      1. Melanin is the major pigment in human skin and hair. Melanin co-purifies with nucleic acid during extraction. Due to its ability to reversibly bind thermostable DNA polymerase, it can be a potent inhibitor of PCR. Fortunately, the inhibition of the enzyme by melanin can be reversed by diluting solutions of preformed complexes or by adding excess amounts of other proteins such as Bovine Serum Albumin (BSA).
      2. The “BSA Method” was developed to address inhibition by melanin, hemoglobin, and other interfering substances. For some tissue samples, diluting the eluate (See appropriate nucleic extraction method) with BSA prior to downstream applications may be effective in eliminating PCR inhibition caused by melanin.
2. **REAGENT:**
   1. Bovine Serum Albumin (BSA), Molecular Biology Grade, 20 mg/ml – Fisher Scientific cat# FERB14. Store at -20°C until expiration date.
3. **PROCEDURE FOR DILUTION:**
   1. If you suspect a PCR inhibitor is present in a sample, you may try the following options:
      1. Blood/Bone Marrow Aspirate/Body Fluids
         1. Re-extract sample and run an extra AW2 wash before eluting off the column. The final wash before the elution step should be clear.
         2. Run an inhibitor study using a 50:50 mix of patient sample and positive control, keeping the total sample input volume the same. (Ex: a 5ul sample volume would have 2.5ul each of patient sample and positive control).
         3. If internal controls fail on an assay, testing may be repeated after diluting with water (for example at a 1:2 or 1:4 ratio). See a Director/Pathologist for further instruction.
      2. Formalin-fixed, paraffin-embedded and other tissues
         1. If the concentration and volume is high, use the Genomic DNA Clean and Concentrate procedure.
         2. Re-extract the sample using the organic extraction protocol found in the TNA, Tissue Manual Extraction Procedure.
         3. If internal controls fail on an assay, testing may be repeated after diluting with water (for example at a 1:2 or 1:4 ratio). See a Director/Pathologist for further instruction.
4. **PROCEDURE FOR BSA METHOD:**
   1. The following procedure (BSA Method) may be used for formalin-fixed, paraffin-embedded (FFPE) tissue and other specimens with melanin pigment or other PCR inhibitors.
      1. If heavy melanin pigmentation, blood, or other PCR inhibitor is identified in a sample, consult Director or Pathologist to determine whether the standard protocol or the BSA Method should be utilized.
      2. If extraction is performed and PCR inhibition is identified, consider utilizing the BSA method prior to additional testing.
   2. BSA method:
      1. Perform extraction per standard procedure. Make a 1:10 dilution of “BSA to Sample” after extraction and prior to Assay set-up.
         1. Add 9ul of DNA sample to a 2ml PCR tube.
         2. Then, add 1ul of BSA (total of 20ug) to the PCR tube.
         3. Mix thoroughly by pipetting up and down.
         4. **Note:** Make enough aliquot to cover the appropriate number of reactions. However, excess 1:10 dilution can be stored at -20°C for later use (label tube accordingly).
      2. Set-up Assay reactions per standard procedure using diluted sample.
      3. If the reaction remains inhibited by melanin, further dilution of the sample with BSA may be needed. See Director/Pathologist for additional instructions.
         1. For example, make a new “BSA to Sample” dilution at 1:5 or at 1:2 (BSA:Sample), and then repeat testing.
         2. **Note:** For heavily pigmented specimens, a 1:2 “BSA to Sample” dilution factor may be required.
         3. If reaction continues to be inhibited, please consult with Lab Director/Pathologist.
5. **REFERENCES:**
   1. Eckhart L, Bach J, Ban J, Tschachler E. Melanin binds reversibly to thermostable DNA polymerase and inhibits its activity. Biochem Biophys Res Commun. 2000 May 19;271(3):726-30.
   2. Giambernardi TA, Rodeck U, Klebe RJ. Bovine Serum Albumin Reverses Inhibition of RT-PCR by Melanin. Biotechniques. 1998 Oct;25(4):564-6.
   3. Profiles in DNA: An Introduction to PCR Inhibition. www.promega.com, March 2007
6. **REVISIONS:** 
   1. 1/15/2020: Previously called the DNA Modification BSA Method, we incorporated Interfering substances from an appendix into the newly labeled procedure. We also updated the footer to include the new laboratory name.
   2. 10/24/2023: Updated the reagent to reflect the switch from NEB BSA to Fisher Scientific BSA.