Using Soil DNA Kit (D5625) to Clean Up DNA containing inhibitors

1. **Vigorously mix the bottle of the HTR reagent for 30 seconds to ensure the particles are throughly resuspended. Add 100µl of HRT reagent to 200 µl of sample and mix throughly by vortexing for 10 seconds.**

   Important: HTR reagent must be throughly suspended before being dispense from bottle**. Tip:** Use 1ml pipettor and cut off the end of 1ml tip to make it easier for pipetting the HTR reagent.

2. Incubate at room temperature for 2 minutes.

3. **Centrifuge at full speed (>13,000 x g) for 2 minutes.**

4. **Transfer cleared supernatant to a new 1.5 ml tube. Note:** If the supernatant still shows dark color from soil at this point, perform the HTR extraction again by repeating step 1-4.

5. **Optional: If RNA-free DNA is required, add 2µl RNase A (25mg/ml) and mix throughly by vortexing for 10 seconds.** Incubate at 37°C for 10 minutes.

6. Add 100µl of equilibration buffer to HiBind DNA Column(inserted into a 2 mL collection tube) and incubate for 4 minutes. Centrifuge at max speed for 30 seconds and discard flowthrough.

7. **Add equal volume of XP2 buffer to the cleared lysate, mix the sample throughly by vortexing**.

8. **Apply entire sample including any precipitation that may have formed, to a HiBind® DNA column assembled in a 2 mL collection tube (supplied)**. Centrifuge at full speed (>13,000 x g) for 1 min at room temperature. Discard flow-through liquid and re-use collection tube.

9. **Place HiBind® DNA column back into the same collection tube and add 300µl of XP2 Buffer.** Centrifuge at full speed (>13,000 x g) for 1 min at room temperature. Discard flow-through liquid and re-use collection tube.

10. **Place the column into a new 2 mL collection tube (supplied) and add 700 µL SPW Wash Buffer**. Centrifuge at full speed (>13,000 x g) for 30 seconds. Discard the flow-through and re-use collection tube.

Note: SPW Wash Buffer is provided as a concentrate and must be diluted with absolute ethanol as indicated on the bottle and page 4. If refrigerated, the diluted SPW Wash Buffer must be brought to room temperature before use.

11. **Add another 700 µL SPW Wash Buffer to the column.** Centrifuge at full speed (>13,000 x g) for 1 minute. Discard flow-through liquid and re-use collection tube in next step.

12. **Discard liquid and re-insert the column to the empty collection tube, centrifuge the column at 14,000 x g for 2 min at room temperature**. ***This step is critical in removing traces of ethanol that will interfere with downstream applications.***

13. **Place column into a clean 1.5 mL microcentrifuge tube (not supplied)**. To elute DNA add 30 µL-50 µL of Elution Buffer (10 mM Tris buffer, pH 8.5) directly onto the center of HiBind® matrix. Incubate at 65°C for 5 minutes.

14. Centrifuge at full speed (>13,000 x g) for 1 min to elute DNA.

15. **Repeat elution step with a second 30 µL-50 µL Elution Buffer**.

Using HTR Reagent to Clean Up DNA Samples with PCR Inhibitors

1. **Vigorously mix the bottle of the HTR reagent for 30 seconds to ensure the particles are throughly resuspended**. **Add 100µl of HRT reagent to 200 µl of sample and mix throughly by vortexing for 10 seconds.**

   Important: HTR reagent must be throughly suspended before being dispense from bottle**. Tip:** Use 1ml pipettor and cut off the end of 1ml tip to make it easier for pipetting the HTR reagent.

2. Incubate at room temperature for 2 minutes.

3. **Centrifuge at full speed (>13,000 x g) for 2 minutes.**

4. **Transfer cleared supernatant to a new 1.5 ml tube. Note:** If the supernatant still shows dark color from soil at this point, perform the HTR extraction again by repeating step 1-4.

5. Add 1 volume of isopropanol and mix by inverting 5-10 times

6. Centrifuge at 13,000 x g for 10 minutes to pellet DNA. Carefully decant the supernatant without disturbing the pellet

7. Wash the pellet in 70% ethanol.
8. Centrifuge at 13,000 g for 10 minutes. Carefully decant the supernatant without disturbing the pellet.
9. Let the pellet air dry for 5-10 minutes and resuspend in water or TE Buffer.