**Spekboom HMW DNA Extraction Protocol**

**Updated October 2022**

*This protocol was used for the initial Spekboom project but we have recently improved our Spekboom HMW extraction protocol – Please see “Spekboom HMW DNA Extraction\_Adapted Cytiva Nucleon PhytoPure Protocol”.*

**Reagents:**

* Sorbitol wash (D-Sorbitol 0.35 M, PVP-40 000 1%(w/v), Tris-HCl pH8 100 mM, EDTA pH8 5 mM) (from Jones and Schwessinger, 2020)
* DTT (1,4-dithiothreitol).
* CTAB 2X (Tris 10 mM pH8.0; EDTA 20 mM, pH 8.0; CTAB 2; NaCl 1.4 M, optional: add 500uL Proteinase K (20 mg/mL) per 10 mL CTAB buffer (from Melino et al., 2022).
* Chloroform.
* Nucleon PyhtoPure DNA extraction resin suspension (Cytiva Nucleon Phytopure Genomic DNA Extraction Kit).
* RNAse A.
* Isopropanol.
* 70% Ethanol.
* Nuclease free dH2O.

***Sampling and Sorbitol Wash***

***Sampling:***

1. Pulverize 1g of spekboom tissue using liquid nitrogen. (Grind for at least 25 min. Inner stem tissue requires longer grinding than leaf tissue). Transfer 1 g of tissue to a 30mL Nalgene extraction tube.

***Sorbitol Wash:***

1. Prepare the sorbitol wash solution (D-Sorbitol 0.35 M, PVP-40 000 1%(w/v), Tris-HCl pH8 100 mM, EDTA pH8 5mM) as per Jones and Schwessinger, 2020. Prepare 100 mL sorbitol wash per 1g tissue prepared for extraction. Add a tiny scoop of DTT to the sorbitol wash solution just before use.
2. Resuspend 1 g of ground tissue in 15 mL sorbitol wash solution. Make sure the tissue is homogenised in the sorbitol solution via shaking or vortexing if necessary. Centrifuge at 5000g for 5 min. Pour off the supernatant and repeat step 3 until supernatant is clear (minimum of 5 x for spekboom leaves).
3. During the final sorbitol wash, resuspend the tissue in exactly 10 mL of sorbitol wash solution. Once tissue is homogenous in solution, aliquot 1mL of tissue using wide bore pipette tips in sorbitol solution to each 2 mL DNA LoBind Tube (Prepare ten 2 mL DNA Lobind Tubes per 1g tissue prepared for extraction). Centrifuge at 5000g for 5 min, remove the supernatant.

***Cell Lysis:***

1. Preheat CTAB 2X at 65°C for 5 min prior to extraction. Resuspend the pellet with 700 μl CTAB 2X (Tris 10 mM pH8.0; EDTA 20 mM, pH 8.0; CTAB 2; NaCl 1.4 M, optional: add 500uL Proteinase K (20 mg/mL) per 10 mL CTAB buffer (concentration from Melino et al., 2022).
2. Heat samples in the water bath to 65°C for 30 min, mixing every 10 min.
3. Remove samples from waterbath and gently invert to tubes 3X to get everything into solution. Allow to cool to 37 °C. Add 7uL RNAse A (20 mg/mL) per sample and leave at room temperature for 10 min.
4. Place samples at 4 °C for 15 min.

***DNA Extraction:***

1. Add 500 uL of ice-cold chloroform slowly starting with drops to prevent cold shock to samples. Mix well by inversion.
2. Add 100 uL of Nucleon Pyhtopure DNA extraction resin suspension. Shake well to get everything homogenous, place in eppi rack with lid and invert rack (gently shake) at room temperature for 10 min. Centrifuge for 10 min @1300g.
3. Transfer the aqueous phase using wide bore pipette tips to a 1.5 mL DNA LoBind Eppendorf tube.
4. Repeat step 9, 10 and 11.
5. Add an equal volume of isopropyl alcohol previously cooled to -20 °C. Mix gently by inversion for 1 min. Place the samples at -20 °C. for 1h. (If a sufficient amount of DNA strings are visible immediately after mixing proceed with centrifuge step).
6. Centrifuge for 5 min at 4000 g (place eppis with back of tube facing to the back of the rotor to help locate pellet). Discard the supernatant and place upside down on tissue paper to remove most of the isopropanol.
7. Resuspend the pellet in 1 mL of ethanol (70%) previously cooled to − 20°C. Mix gently by inverting tubes 5x.
8. Centrifuge for 5 min at 4000 g (place eppis with back of tube facing to the back of the rotor to help locate pellet). Discard the supernatant gently and place upside down on clean tissue paper to remove most of the ethanol.
9. After a quick spin down, immediately take off excess ethanol with a pipette. Allow to dry for max 30 seconds.
10. Resuspend the pellet in 20 μL of dH20 (or preferred buffer for sequencing library preparation).
11. Heat to 50°C for 15 min if pellet does not dissolve sufficiently.
12. Proceed to QC DNA. Do not freeze DNA, store at 4 °C.

Link to sorbitol wash solution:

Ashley Jones, Benjamin Schwessinger 2020. Sorbitol washing complex homogenate for improved DNA extractions. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.beuvjew6>