A METHOD OF EXTRACTING DNA SUITABLE FOR PCR FROM VEGETABLE OIL SAMPLES

1. SCOPE AND FIELD OF APPLICATION

This method is suitable for the extraction of DNA from vegetable oil samples. Although the quantity of DNA recovered is low it is suitable for down-stream applications involving PCR.

2. REFERENCES

Promega Technical Bulletin No 284 (Part N^{o}: TB284).

3. PRINCIPLE OF METHOD

Cellular material, including DNA fragments, present in an oil sample are collected as a pellet by centrifugation at 20-25,000g. DNA is then extracted from this pelleted material using the Promega Wizard® Magnetic DNA Purification System for Food.

The Promega Wizard® Magnetic DNA Purification System for Food is designed for DNA extraction from foodstuffs including oils. The DNA Purification System utilises MagniSil™ Paramagnetic Particles (PMPs) to aid in DNA extraction and purification.

Under extraction conditions the PMPs bind DNA present in solution but do not bind proteins or other cellular material. The PMPs can be considered as a fluid, solid-phase extraction medium. This allows DNA to bind to the magnetic particles in solution and thus increases binding efficiency. The PMPs can be captured using a magnet which permits the removal of wash solutions without loss of DNA. The PMPs can be completely resuspended during wash steps which enhances the removal of contaminants from samples thus improving the purity of final DNA extracts. DNA is finally eluted from the PMPs using water or TE buffer.

4. EQUIPMENT

- Gilson pipettes.
- Benchtop whirlimixer.
- PolyATtract® System 1000 Magnetic Separation Stand (Promega Cat#: Z5410).
- High-speed centrifuge capable of exerting over 20,000g, e.g. Sorvall RC-5B.
- Bench top centrifuge with rotor for 50ml Falcon tubes.
- Micro-centrifuge.
- Rotary mixer.
- 50ml Falcon tubes with screw caps.

The following items are sterilised by autoclaving:

1.5 ml Eppendorf tubes.
50ml screw cap polypropylene centrifuge tubes for use in Sorvall centrifuge.
250ml screw cap polypropylene centrifuge tubes for use in Sorvall centrifuge.

5. REAGENTS

All reagents should be of a suitable purity defined for molecular biology analysis (e.g. Sigma Molecular Biology products). The water used should be sterile Milli-Q water or equivalent purity.

- 70% (v/v) Ethanol

- Isopropanol.

- Promega Wizard Magnetic DNA Purification System for Food

Kits are available in 200 assay (Cat: FF3750) or 400 assay (Cat: FF3751) sizes.

Single components can be purchased separately:

- RNase A solution (4mg/ml) (Cat: A7973)
- Lysis Buffer A (Cat: A8191)
- Lysis Buffer B (Cat: Z3191)
- Precipitation Solution (Cat: Z3201)

- HazTab Solution

Dissolve two HazTab tablets in 1 litre of water. This provides 5,000ppm chlorine.

6. PROCEDURE

Sufficient DNA can be extracted from 200ml of oil to allow about 6 PCR reactions (5µl of template DNA) to be performed.

Centrifugation of Oil Samples

Centrifuge tubes (250ml) should be of high strength polypropylene material suitable for use at high speeds (>20,000g). Tubes should be the screw cap type to prevent spills and cross-contamination.

Tubes should be clean and free from contamination. Polypropylene tubes can be autoclaved, however, remove lids before autoclaving to prevent damage.

Clean (washed) tubes should be rinsed with HazTab solution to degrade residual DNA material then rinsed with 3 lots of distilled water. Tubes should then be rinsed with 70% ethanol followed by a last wash with sterile distilled water (SDW). Finally tubes should be autoclaved with lids removed to prevent damage.

Oil samples should be dispersed into autoclaved 250ml tubes. Each tube should be filled with about 200ml of oil. Replace caps on tubes.

An extraction negative tube filled with 200ml of Milli-Q water should be included with each extraction set.

Tubes should be matched in pairs for centrifugation. Each pair of tubes should be balanced to within ±1.0g.

Note: If there are sufficient samples to fill the rotor, the extraction negative (tube filled with 200ml Milli-Q water) can be left to stand for 1 hour on the bench.

Place balanced tube pairs into opposing slots in the centrifuge rotor. Ensure the rotor is balanced and that there are no single tubes (without a balance) present.

Centrifuge the oil samples for 1 hour at >20,000g.

DO NOT EXCEED MAXIMUM SPEED FOR ROTOR.

When centrifuge has stopped carefully remove tubes from rotor and decant upper oil layer into a waste container leaving the pellet at the base of the centrifuge tube.

Use a marker pen to mark the outer edge of tube so pellet can be readily located in following steps. This is especially useful if the pellet size is small.

Extraction of DNA from Pellet

DNA is extracted from the pelleted material using the Wizard® Magnetic DNA Purification System For Food.

Add 2.5ml of Lysis Buffer A and 25µl of RNase A solution to tube. Replace cap and vortex to resuspend the pellet. It may be necessary to use a Pasteur pipette to fully dissolve pellet.

Add 1.25ml of Lysis Buffer B and vortex for 10 seconds to mix. Lay tube on rotary mixer and incubate for 10-30 minutes at room temperature.

Add 3.75ml of Precipitation Solution (blue colour) and vortex vigorously to mix. Break up any lumps using a pipette tip. The solution should be a greeny-blue colour.

Transfer the entire content of the 250ml centrifuge tube to a 50ml centrifuge tube. Centrifuge solution for 10 minutes at 3,000-5,000g.

Use a narrow, plastic Pasteur pipette to transfer supernatant (lower, greeny-blue liquid phase) to a fresh 50ml falcon tube. Avoid transferring any of the solid material or upper layer. If solution is not clear repeat centrifugation and transfer step.

Vigorously shake MagniSil™ PMPs to resuspend. Use a pipette to add 100-150µl to the supernatant and vortex vigorously to mix.

Estimate total volume of solution (use scale on side of 50ml tube) and add 0.9 volumes of isopropanol. Invert tube 20 times to mix, then incubate for 5 minutes at room temperature with occasional mixing.

Place tube in a PolyATtract System Magnetic Separation Stand for 1 minute to capture magnetic beads. While maintaining magnetic attraction remove liquid phase to waste.

Remove tube from magnet and add 1.25ml of Lysis Buffer B. Mix well and wash beads and whole inside surface of tube with buffer.

Place tube in the PolyATtract Magnetic Stand for 1 minute, to capture beads, and remove liquid as above.

Remove tube from magnet and resuspend beads in 5ml ±0.5ml of 70% ethanol. Place tube in the PolyATtract Magnetic Stand for 1 minute and remove and discard liquid as above.

Repeat wash steps twice for a total of 3 washes. After the final wash add 1.0ml of 70% ethanol to the beads. Wash the beads into the bottom of the tube and resuspend them by aspiration with a pipette.

Transfer the whole solution and suspended beads to a clean 1.5ml Eppendorf tube. Place the tube in a small PolyATtract Magnetic Stand for 1 minute. Remove and discard as much liquid as possible using a P200 Gilson.

Air dry beads at room temperature (approximately 22ºC) for 20-30 minutes or at 60ºC to 65ºC for 10 minutes. Do not dry to completion.

When beads have dried add 50µl of sterile distilled water to the beads. Resuspend the beads by vortexing gently for 30 seconds.

Incubate at 4ºC overnight to elute DNA. If DNA is required immediately incubate tube at 65ºC for 5 minutes.

This is the final DNA solution. The volume of DNA recovered is generally too low to quantify, however, it is suitable for PCR.

Note: The beads can be added directly to PCR reactions; however, they should not be used if they have been frozen (<0ºC).

Cleaning of Equipment

Clean centrifuge rotor in warm soapy water using a soft brush or cloth to remove any dirt or oil spills. Rinse rotor in distilled water and dry.

Clean the centrifuge bowl using warm soapy water to remove any spilt materials. Dry the bowl with paper towels and rinse with 80% ethanol.

Centrifuge tubes can be cleaned in a dishwasher or using warm soapy water followed by distilled water. Tubes can be prepared for re-use or store as required.

7. RISK ASSESSMENT

Before performing this protocol, ensure you have read and signed the appropriate risk assessment forms.