B66720AC
November 2021

Chloropure Kit

Nucleic Acid Extraction from Plants

Please refer to www.beckman.com for updated protocols. For questions regarding this protocol, call Beckman Coulter Technical Support at 1-800-369-0333.

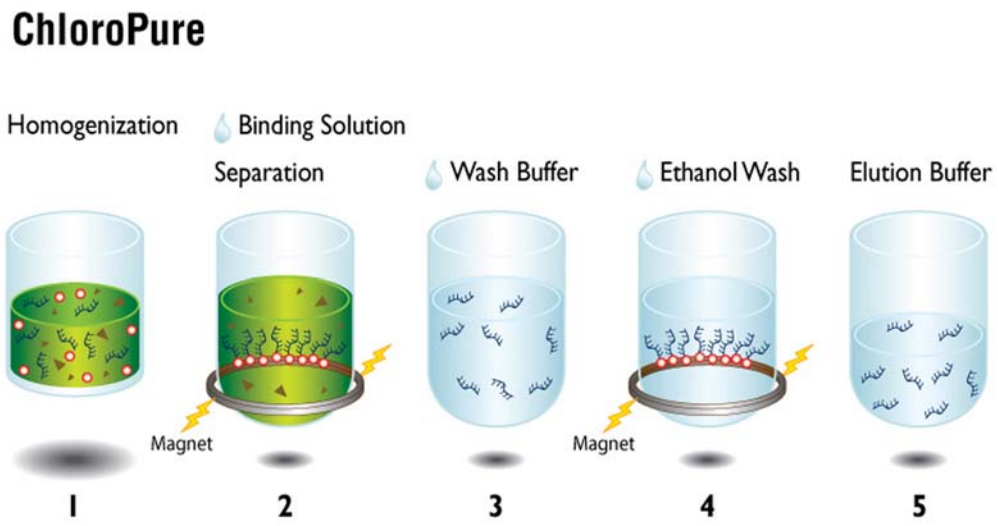
Agencourt Chloropure is intended for molecular biology research applications. It is not intended or validated for use in the diagnosis of disease or other conditions.

Table of Contents

Introduction

The Chloropure kit utilizes the patented SPRI paramagnetic bead-based technology to isolate nucleic acids from a maximum input of three 6mm lyophilized leaf punches or 40 mg ground seeds or fresh leaf material. The protocol can be performed in 96-well plates (manually and fully automated). Extraction begins with adding the lyophilized plant material directly to the lysis buffer. Binding buffer is then added to facilitate immobilization of the nucleic acids to the surface of paramagnetic beads. The contaminants are rinsed away using a simple washing procedure. The Chloropure procedure does not require vacuum filtration or centrifugation.

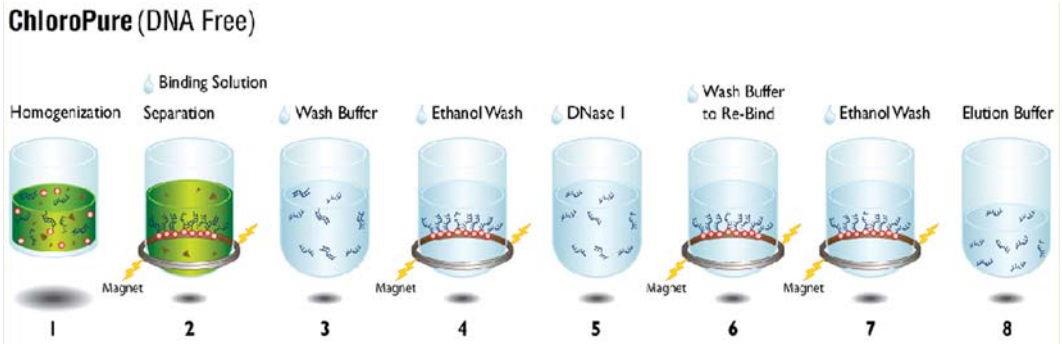
Process Overview: Total Nucleic Acid Extraction



The Chloropure procedure for the extraction of total nucleic acid, is performed in the following stages:

1. Homogenization of plant material
2. Binding of nucleic acids to magnetic beads and separation
3. Washing of captured nucleic acids with wash buffer
4. Washing with 70% ethanol to remove contaminants
5. Elution of nucleic acids from the magnetic particles

Process Overview: RNA Only Extraction (DNA Free)



The Chloropure procedure for the extraction of total nucleic acid, is performed in the following stages:

1. Homogenization of plant material
2. Binding of nucleic acids to magnetic beads and separation
3. Washing of captured nucleic acids with wash buffer
4. Washing of beads with 70% Ethanol
5. DNase I
6. Addition of Wash Buffer to Rebind the RNA
7. Washing with 70% ethanol to remove contaminants
8. Elution of total RNA from the magnetic particles

Warnings and Precautions

DANGER	
Lysis: Guanidine Thiocyanate 10-20%	
Polyoxyethylated Octyl Pheno <1%	
H303	May be harmful if swallowed.
H314	Causes severe skin burns and eye damage.
P280	Wear protective gloves, protective clothing and eye/face protection.
P303+P361+P353	IF ON SKIN (or hair): Rinse skin with water.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310	Immediately call a POISON CENTER or doctor/physician.
SDS	Safety Data Sheet is available at beckman.com/techdocs.

	**DANGER**	
	Wash: Guanidine Thiocyanate 5-10%	
	Polyoxyethylated Octyl Pheno <1%	
	**H303**	May be harmful if swallowed.
	H314	Causes severe skin burns and eye damage.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P303+P361+P353	IF ON SKIN (or hair): Rinse skin with water.
	P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P310	Immediately call a POISON CENTER or doctor/physician.
	SDS	Safety Data Sheet is available at beckman.com/techdocs.

Materials Supplied in the Kit

Reagent	Storage Conditions on Arrival	Storage Conditions once In Use
Chloropure Lysis Buffer	Room Temperature	Room Temperature
Chloropure Bind Buffer	Room Temperature	Prepare Fresh
Chloropure Wash Buffer	Room Temperature	Room Temperature

Materials Required but not Provided

Consumables and Hardware

- **Agencourt SPRIPlate 96R - Super Magnetic Plate** (Beckman Coulter product # A32782)
- **For Manual Digestion/Processing** 1.2 mL plates (Thermo Scientific product # AB1127)
- **For Automated Digestion/Processing** 2.2 mL Ritter Deepwell plates (World Wide Medical product # 99181000)
- **Adhesive plate film** (Thermo Scientific product # AB-0558)
- **Destination Plate:** 300 µL round bottom microtiter plate (96-well (300 µL well capacity) round bottom plate. [Costar product # 07-200-105])
- **Liquid handling robotics or a hand pipette**
- **37°C water bath (not required for total nucleic acid extraction)**

Reagents

- **100% Isopropanol, ultra pure** (American Bioanalytical product # AB-07015)
- **Fresh 70% ethanol made with nuclease free water** (American Bioanalytical product # AB-00138)

  NOTE Note: 70% ethanol is hygroscopic. Fresh 70% ethanol should be prepared for optimal results.

- ***For RNA Only Extraction:*** **DNase I (RNase-free)** [(2U/µL); Ambion Inc. product # AM2222]
- ***For RNA Only Extraction:*** **DNase I 10X buffer** (Ambion Inc. product # AM8170G)
- **Reagent grade water, nuclease-free** (Ambion Inc. product # AM9932)

Chloropure Procedure

Assembly Step 1 is performed once for each new Chloropure kit. If you have already made the following preparations for a previous experiment, please skip ahead to Step 2.

1. **Add 80 mL of 100% Isopropanol to the Wash Buffer bottle.**

   After addition of Isopropanol invert the bottle to mix. Once the solution has been thoroughly mixed, store at room temperature.

2. **Prepare Bind Buffer:**

   Combine 6 µL Bind Buffer with 150 µL of 100% isopropanol for each individual isolation in an nuclease free vessel of suitable size (for example: for 10 isolations, add 60 µL of Bind Buffer to 1.5 mL of 100% isopropanol in a 15 mL conical tube).

   Vortex Bind Buffer bottle thoroughly before combining. Unused combined solution should be discarded.

   FOR RNA ONLY EXTRACTION:

3. **Prepare DNase solution. Prepare this solution fresh for each isolation — discard any unused solution.**

   Combine 20 µL nuclease free water, 2.5 µL 10X DNase buffer, and 2.5 µL of DNase I.

4. **Homogenize each sample in 300 µL of Lysis Buffer. (sample input should not exceed three 6 mm lyophilized leaf punches or 40 mg ground seeds or fresh leaf material).**

   General Homogenization Recommendations: Lyophilized leaf punches or fresh leaf material may be added directly to the Lysis Buffer and homogenized in any commercially available bead mill (ie. GenoGrinder 2000). Seed material may need to be preground before addition of the lysis buffer. Optimal homogenization methods and sample input amount need to be determined experimentally, according to thickness of leaf/seed. Homogenization may be scaled up to any volume using this ratio — although only 150 µL of lysate may be processed per well in a 96-well plate. Lysate may be frozen indefinitely at -80°C.

5. **Centrifuge lysate for 10 minutes at 1100 RCF to pellet debris.**
6. **Transfer 150 µL of homogenized lysate to 1.2 mL processing plate.**

7. **Pipette 150 µL Bind Buffer (prepared in step 2), mix by slowly pipetting 5 times and incubate at room temperature for 5 minutes.**

   Aspirate from the top down to avoid disturbing the pellet

8. **Move the plate onto the Supermagnet and separate for 2-4 minutes.**

   Wait for the solution to clear before proceeding to the next step.

9. **Slowly aspirate the cleared solution from the plate and discard.**

   This step must be performed while the plate is situated on the magnet. Do not disturb the separated magnetic beads. If beads are drawn out, leave a few microliters of supernatant behind.

10. **Remove the plate from the magnet and add 300 µL of Wash buffer. Pipette mix 10 times and incubate for 1 minute.**

11. **Return plate to the magnet and separate for 2-4 minutes.**

    Wait for the solution to clear before proceeding to the next step.

12. **Slowly aspirate the cleared solution from the plate and discard.**

    This step must be performed while the plate is situated on the magnet. Do not disturb the separated magnetic beads. If beads are drawn out, leave a few microliters of supernatant behind.

    FOR TOTAL NUCLEIC ACID EXTRACTION: Skip to step 22.

    FOR RNA ONLY EXTRACTION:

13. **Remove the plate from the magnet and add 300 µL of 70% ethanol. Pipette mix 10 times to resuspend the beads.**

14. **Return the plate to the magnet and separate for 2 minutes.**

    Wait for the solution to clear before proceeding to the next step.

15. **Slowly aspirate the cleared solution from the plate and discard.**

    This step must be performed while the plate is situated on the magnet. Do not disturb the separated magnetic beads. If beads are drawn out, leave a few microliters of supernatant behind.

16. **Remove the plate from the magnet and 25 µL DNase solution as prepared in step 3.**

17. **Pipette mix 5 times to resuspend the beads in the DNase solution.**

18. **Seal and incubate plate in a 37°C water bath for 15 minutes to facilitate digestion of DNA.**

19. ***DO NOT REMOVE THE DNase SOLUTION.* Add 138 µL Wash Buffer and pipette mix 10 times. Incubate at room temperature for 5 minutes.**

20. **Place plate onto the magnet and separate for 2-4 minutes.**

    Wait for the solution to clear before proceeding to the next step.

21. **Slowly aspirate the cleared solution from the plate and discard.**

    This step must be performed while the plate is situated on the magnet. Do not disturb the separated magnetic beads. If beads are drawn out, leave a few microliters of supernatant behind.

22. **Remove the plate from the magnet and add 300 µL of 70% ethanol. Pipette mix 10 times to resuspend the beads.**

23. **Return plate to the magnet and separate for 2 minutes.**

Wait for the solution to clear before proceeding to the next step.

24. **Slowly aspirate the cleared solution from the plate and discard.**

    This step must be performed while the plate is situated on the magnet. Do not disturb the separated magnetic beads. If beads are drawn out, leave a few microliters of supernatant behind.

25. **Repeat steps 22-24 for a total of 2 ethanol washes.**

26. **Let the plate air dry for 5 minutes at room temperature.**

    The plate should air-dry until the last visible traces of ethanol evaporate. Over-drying the sample may result in a lower recovery.

27. **Remove the plate from the magnet and add 50 µL of nuclease-free water. Resuspend the beads by pipette mixing 10 times.**

    Smaller or larger elution volumes can be used for more or less concentrated product, however the minimum elution volume should be 40 µL to ensure complete elution. Optimal elution volumes need to be experimentally determined, higher yielding plants require larger elution volumes.

28. **Incubate the plate at room temperature for 2 minutes.**

29. **Place the plate on the magnet for 2 minutes and transfer 35 µL eluted nucleic acid to a suitable 96-well storage plate.**

    Wait for the solution to clear before transferring sample.

Revision History

Initial Issue AA, 1/2015

Revision AB, 9/2019

Revision AC, 11/2021

- Updates were made to the following: Added the UK address and phone number to page 8.

Product Availability

Chloropure
REF A47949 - Chloropure, 384 Prep Kit
REF A47951 - Chloropure, 9600 Prep Kit

Trademarks

Beckman Coulter, Inc.
250 S. Kraemer Blvd.
Brea, CA 92821 U.S.A.

Contact Us
If you have any questions, contact our Customer Support Center.

- Worldwide, find us via our website at www.beckman.com/support/technical
- In the USA and Canada, call us at 1-800-369-0333.
- Outside of the USA and Canada, contact your local Beckman Coulter Representative.

May be covered by one or more pat.— see www.beckman.com/patents

Glossary of Symbols is available at beckman.com/techdocs (PN C05838)

Beckman Coulter (UK) Ltd.
Oakley Court
Kingsmead Business Park, London Road
High Wycombe
United Kingdom HP11 1JU
01494 441181