Twist DNA Purification Kit Protocol

For use with the Twist NGS Workflow

The Twist DNA Purification Kit is intended for easy, rapid, and efficient DNA extraction from agrigenomic sample types such as plant and mammalian tissue samples. The excellent yield and purity of the DNA obtained allow for seamless use with the Twist FlexPrep UHT Library Preparation Kit.

This manual details the steps for extracting and purifying DNA from plant or animal tissue. This protocol should only be performed with reagents specified or their equivalents.

Twist NGS Workflow. *The complete NGS workflow takes you from sample preparation to NGS sequencing and data analysis.*

For Research Use Only. *Not intended for use in diagnostic procedures.*

PROTOCOL COMPONENTS

Please read the product packaging and storage recommendations carefully for each kit and store components as recommended immediately upon arrival.

CATALOG #	NAME	DESCRIPTION	STORAGE
TWIST DNA PURIFICATION KIT			
127373 (192 samples) 127375 (1152 samples)	Twist DNA Purification Kit (4C)	· Protease · RNAse · Purification Plate	4°C
		· Low-TE Buffer · Lysis Buffer · Clearing Solution	2-25°C
127372 (192 samples) 127374 (1152 samples)	Twist DNA Purification Kit (Ambient)	Plates & Seals	Room Temperature

For catalog numbers that include DNA extraction with library preparation and hybridization components:

127857: Twist FlexPrep UHT Pure Ag DNA LP Kit, 192 Samples
127858: Twist FlexPrep UHT Pure Ag DNA LP and Hyb Kit, 192 Samples
127859: Twist FlexPrep UHT Pure Ag DNA LP Kit, 1152 Samples
127861: Twist FlexPrep UHT Pure Ag DNA LP and Hyb Kit, 1152 Samples

LEGAL

This document may contain references to other third-party resources such as sources of information, hardware or software, products, or services and/or web sites owned or licensed by third parties. Twist Bioscience does not control or take responsibility for any third-party resources, including, without limitation, the accuracy, reliability, copyright compliance, compatibility, performance, legality, or any other aspect of third-party resources. The inclusion of such resources in this document does not imply endorsement by Twist Bioscience of any third-party resources.

Certain processes described in this document may be subject to patent rights or licenses in local jurisdictions, including those owned or licensed by parties other than Twist Bioscience. Purchase of this product does not include a license to perform any such processes. Users of this product may, therefore, be required to obtain a patent license depending upon the particular application and country in which the product is used before performing such processes.

Twist Bioscience, the various Twist logos, and the Twist NGS logo used herein are trademarks of Twist Bioscience Corporation. All other trademarks are the property of their respective owners.

INTENDED USE

This product is for research use only. This product is not intended for the diagnosis, prevention, or treatment of a disease or condition. Twist Bioscience assumes no liability regarding use of the product for applications in which it is not intended.

TABLE OF CONTENTS

MATERIALS SUPPLIED BY USER

The following materials or their equivalent are required to extract DNA using the Twist DNA Purification Kit.

PRODUCT	SUGGESTED SUPPLIER
REAGENTS AND CONSUMABLES	
Conditioning Plate	FISHERBRAND (12-566-611)
Sterile Steel Beads (OPTIONAL)	Qiagen (69989)
Lysis Plate	FISHERBRAND (12-566-611)
EQUIPMENT	
15-ml and 50-ml Falcon tubes for master mixes	—
Multichannel reagent reservoir	—
Plate centrifuge (SBS format, at least 4500 x g, hold plate stacks of 5 cm height, swing-out rotor)	—
Pipettes and tips	—
Thermal shaker for plates	Eppendorf (C Thermomixer)
Vortex mixer	—

GENERAL NOTES AND PRECAUTIONS

Wear appropriate protective equipment (lab coat, gloves, and protective glasses or goggles) at all times when performing this protocol. For best results, read this document before performing the protocol and follow the instructions provided. Twist cannot guarantee the performance of the Twist DNA Purification Kit if modifications are made to the protocol.

The purification matrix within the Purification Plate must be kept undamaged to avoid short-circuit currents. Short-circuit currents result in the introduction of lysis components into the eluate and inadequate purification, which can affect downstream analysis. Therefore, when applying the lysate to the matrix, avoid touching the surface of the filter matrix and pipette slowly.

For proper handling of the Purification Plate, use the recommended g-force centrifuge settings. Most centrifuges offer the choice between rpm and g-force (rcf); if not, calculate the rpm corresponding to the required g-force using the calculator here: www.geneinfinity.org/sp/sp_rotor.html

We recommend using fresh material or material that has been immediately frozen after sampling and stored at –20 °C or lower. Repeated freezing and thawing of samples should be avoided, since this can reduce DNA fragment size. In general, the use of low-quality starting material results in reduced length and yield of purified DNA.

Check Lysis Buffer for precipitation. If present, warm at room temperature or up to 40°C until clear. Lysis Buffer can be stored at room temperature to avoid precipitation.

It is recommended to store purified DNA in a tightly capped tube at 2–8 °C for 24 hours or at –20 °C for long-term storage.

FOR TECHNICAL SUPPORT, CONTACT CUSTOMERSUPPORT@TWISTBIOSCIENCE.COM.

GUIDANCE FOR KEY STEPS

LYSIS GUIDELINES

Lysis of input material is critical for obtaining sufficient gDNA yields. Optimization may be needed for each sample type to ensure efficient lysis. For plant samples, the efficient mechanical disruption and homogenization of the starting material is critical to the successful extraction of gDNA.

If gDNA yields are low, bead beating can be introduced to mechanically promote lysis. The duration of lysis can also be lengthened from 30 minutes to as long as overnight to complete lysis. Longer lysis incubations can be especially helpful when heated mixing for lysis is not available.

SAMPLE LOADING RECOMMENDATIONS

Sample loading volume per well in the Purification Plate should not exceed 100 µl. If users observe challenging fragmentation (e.g., larger than expected fragments) with the Twist FlexPrep UHT Library Preparation Kit, decreasing the volume loaded into the Purification Plate is a potential solution. Decreasing the volume will decrease the total yield of purified gDNA, but can also improve lysate purity. The FlexPrep UHT Library Preparation Kit does not require high-concentration gDNA, but using high-purity gDNA can improve fragmentation efficiency. Decreasing the sample loading volume to as low as 50 µl can help to achieve high-purity gDNA that is more suitable for the FlexPrep UHT Library Preparation Kit.

PROTOCOL OVERVIEW

This protocol begins with mammalian or plant material and generates purified DNA ready for library preparation.

	DNA EXTRACTION WORKFLOW (PURIFIED gDNA)	TIME
STEP 1	**Lysis** Prepared Samples	**45 minutes***
STEP 2	**Purification** Purified gDNA	**15 minutes**

**Lysis step estimate assumes a 30-minute lysis. If lysis incubation is extended, this step will take longer.*

STEP 1 LYSIS

Source plant or mammalian samples are lysed before performing DNA purification. For guidance on lysis optimization, please refer to the Guidance for Key Steps section above.

Reagents Required

- Plant or mammalian samples
- Sterile steel bead (optional)
- Lysis Plate
- From Twist DNA Purification Kit (4C)
  - Lysis Buffer
  - Protease
- From Twist DNA Purification Kit (Ambient):
  - Adhesive Foils

Before You Begin

- Pre-heat the thermal shaker to 60°C
- Set the centrifuge to 1,000 x g
- Place the Lysis Plate on ice or a cooling block
- Check Lysis Buffer for precipitation. If present, warm at room temperature or up to 40°C until clear. Lysis Buffer can be stored at room temperature to avoid precipitation.

⚠IMPORTANT: Carry out the complete DNA extraction at room temperature.

NOTE: For the centrifuge, choose x g (rcf), not rpm (unless stated otherwise).

LYSIS

1.1

Transfer the sample material into each well of a lysis plate.

NOTE: If possible, cut tissue into small pieces to ensure best lysis conditions.

1.2

OPTIONAL: Add a sterile steel bead into each well of a lysis plate and centrifuge the lysis plate for 1 minute at 1000 x g to collect material at the bottom of each well. For certain tissue types, such as plant tissue, alternative bead beating conditions and further optimization may be necessary.

1.3

Prepare a lysis master mix in one or multiple 50-ml tube(s) as indicated in the table below:

REAGENT	VOLUME*		
	1 SAMPLE	192 SAMPLES	1152 SAMPLES
Lysis Buffer	100 µl	21.21 ml	126.75 ml
Protease	5 µl	1.96 ml	6.34 ml
Total Volume	**105 µl**	**22.18 ml**	**133.96 ml**

**Prepare a master mix for multiple reactions. Volumes listed for 192 and 1152 samples include 10% overage.*

1.4 Add 100 µl of lysis mix to each sample. Seal the lysis plate with Adhesive Foil.

NOTES:

- Make sure that the lysis mix covers the entire tissue sample to ensure complete lysis.
- Alternatively, cap strips can be used to seal the lysis plate.

1.5 Incubate the lysis plate for 30 minutes at 60°C with constant shaking at 2,000 rpm.

NOTES:

- Mixing during 60°C incubation optimizes the lysis efficiency.
- Incubation can be prolonged to increase lysis. Incubation can be extended for several hours and up to overnight to increase lysis efficiency.
- If agitation during incubation is not feasible, mix the lysate before incubation (2000 rpm for 2 minutes). Repeat mixing every 30 minutes over the course of lysis.
- Near the end of this step, start the Purification Plate Preparation section of Step 2: Purification.

1.6 Incubate at 80°C for 10 minutes at 2,000 rpm.

PROCEED IMMEDIATELY TO STEP 2: PURIFICATION

STEP 2 PURIFICATION

This step involves preparing the purification plate and extracting DNA from the lysate prepared in Step 1. For guidance on loading samples onto the purification plate, please refer to the Guidance for Key Steps section above.

Reagents Required

- Lysate from Step 1
- Conditioning Plate
- From Twist DNA Purification Kit (4C)
  - Purification Plate
  - Clearing Solution
  - RNase
  - Low-TE Buffer
- Twist DNA Purification Kit (Ambient)
  - Elution Plate
  - Sealing Foils

PURIFICATION PLATE PREPARATION

2.1 Detach first the lower and then the upper foil from the Purification Plate. Keep the plates in a horizontal position when removing the foils, as the wells contain liquid.

NOTES:

- If the Purification Plate was not shipped or stored upright, the matrix may stick to the upper foil (visible from the site). In this case, vortex the plate until the matrix is removed from the upper foil.
- Make sure the foil is completely removed from the bottom.

2.2 Place the Purification Plate on top of the Conditioning Plate.

NOTE: Conditioning Plates can be reused.

2.3 Centrifuge plate stack for 1 minute at 1,000 x g, then discard flow-through.

NOTE: The centrifuge rotor should be capable of holding plate stacks that have a height of 5 cm.

PURIFICATION

2.4 Place the Purification Plate on top of the Elution Plate.

2.5 Equilibrate samples in Lysis Plate at room temperature for 5 minutes. Remove adhesive foil.

2.6 Prepare a clearing solution master mix in a 15-ml or 50-ml falcon as indicated in the table below

REAGENT	VOLUME*		
	1 SAMPLE	192 SAMPLES	1152 SAMPLES
Cleaning Solution	15 µl	3.17 ml	17.285 ml
RNase	1 µl	211.2 ml	1.27 ml
Total Volume	**16 µl**	**3.380 ml**	**20.275 ml**

**Prepare a master mix for multiple reactions. Volumes listed for 192 and 1152 samples include 10% overage.*

2.7 Add 15 µl clearing solution mix to each sample. Mix thoroughly by shaking or pipetting and incubate for 3 minutes at room temperature.

2.8 Centrifuge the Lysis Plate for 3 minutes at max speed.

2.9 Transfer 100 µl of the cleared lysate from Lysis Plate to the prepared Purification Plate on top of the Elution Plate.

⚠ IMPORTANT:
- Pipette slowly and vertically in the middle of the purification matrix.
- Do not touch the matrix bed with the pipette tip during sample loading.
- See Guidance for Key Steps section above on lowering volume loaded into Purification Plate

2.10 Centrifuge the plate stack (Purification Plate on top of the Elution Plate) for 1 minute at 1,000 x g. Elution plate can be sealed with a Sealing Foil.

2.11 Purified DNA is in the flow-through and ready-to-use.

2.12 Perform QC with spectrophotometric analysis to evaluate sample purity.
NOTE: For spectrophotometric analysis, use the Low-TE Buffer supplied with the kit as a blank.

STOPPING POINT: If not proceeding immediately to library preparation, the extracted DNA can be stored at -20°C.

APPENDIX A: KIT COMPONENTS

The following table details part numbers for each component provided in the kits required for this protocol.

BOX	COMPONENT	COMPONENT PART NUMBER
Twist DNA Purification Kit (4C)	Lysis Buffer	126870 (192 samples) 126875 (1152 samples)
	Protease	126871 (192 samples) 126876 (1152 samples)
	Clearing Solution	126872 (192 samples) 126877 (1152 samples)
	RNAse	126873 (192 samples) 126878 (1152 samples)
	Low-TE Buffer	126874 (192 samples) 126879 (1152 samples)
	Purification Plate	126780
Twist DNA Purification Kit (Ambient)	Elution Plates	126804
	Sealing Foils	126805
	Adhesive Foils	126806

END OF APPENDIX