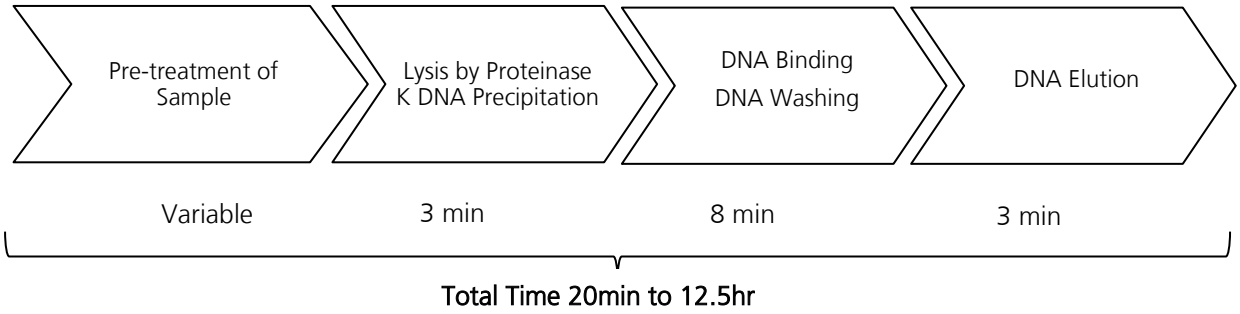
Extracta Plus DNA

Cat. No. 95213-010 Size: 10 reactions **Store at 15°C to 25°**
95213-050 50 reactions

Description

Extracta Plus DNA kits offer rapid purification of total DNA from a variety of samples sources. Genomic, mitochondrial and pathogenic DNA can be collected from fresh/frozen animal cells, tissues, blood, and bacteria. The Extracta Plus Spin Column workflow enables simultaneous processing of multiple samples into purified DNA suitable for PCR, Southern blotting, RAPD, AFLP and next-generation sequencing applications.
Purified DNA is eluted in low salt buffer or water. DNA fragments purified from this kit will be predominately in the 30 kb range, with recovery of fragments as large as 50 kb and as small as 100 bp made possible.

Workflow Overview



Components

	Volume	
Component Description	**10 Reactions**	**50 Reactions**
Proteinase K (1.25 ml/2)	1 x 1.25 ml	1 x 1.25 ml
Buffer EPDTL	1 x 1.8 ml	1 x 14 ml
Buffer EPDL	1 x 2 ml	1 x 12 ml
Buffer EPDW1	1 x 5 ml	1 x 19 ml
Buffer EPDW2	1 x 5 ml	1 x 13 ml
Buffer EPDE	1 x 2 m	1 x 15 ml
Extracta Plus Spin Column	10 piece	50 piece
Collection Tubes 2 ml	20 piece	100 piece

Storage and Stability

Store Extracta Plus Spin Columns and buffers in a dry room temperature (15-25°C) location upon receipt. The supplied ready-to-use Proteinase K solution is stable for at least 1 year at room temperature (15-25°C). If ambient temperatures regularly exceed 25°C, or longer storage is required, Proteinase K solution can be stored at 2-8°C.

Additional reagents and materials that are not supplied

- Ethanol (96–100%)
- Optional: DNase-free RNase A (100 mg/ml)
- For blood and cultured cells
  - PBS, pH 7.2 (50 mM potassium phosphate, 150 mM NaCl)
- For pre-treatment of paraffin-embedded tissue
  - Xylene
- For pre-treatment of formalin-fixed tissue
  - PBS, pH 7.2 (50 mM potassium phosphate, 150 mM NaCl)
- For pre-treatment of gram-positive bacteria
  - Lysozyme lysis buffer (see Appendix B)

General Guidelines

- Best results are obtained with fresh or fresh frozen material. Repeated freeze thaw events should be avoided as this can accelerate DNA degradation.
- DNA quality is impacted by sample type and sample quality. Poor quality starting material will reduce average fragment length and yield of purified DNA.
- After Proteinase K digestion, tissue samples can be stored in Buffer EDTL for up to 6 months at room temperature without impacting DNA quality.
- For bacterial cultures that form very dense cell walls, harvest cells at the early log phase of growth for best results. Fresh or fresh frozen pellets are compatible with this procedure.
- Suitable personal protective equipment should be worn when handling chemicals.
- Read the entire protocol before beginning. Take note of different pre-treatments required for various sample inputs plan your workflow accordingly.

Point in protocol where procedure can be stopped and stored at appropriate conditions outlined

Take note of recommendations in protocol

Use caution to obtain the best results when performing protocol

Before You Begin

- Wipe down work areas and pipettes with an RNase and DNA cleaning product.
- Prepare Buffer EPDW1 and Buffer EPDW2: Before first use, add the appropriate amount of ethanol (96-100%) indicated on the bottle to the buffer concentrate and shake to mix thoroughly. Once ethanol is added, store closed tightly at room temperature for up to 1 year.
- Buffer EPDL may form a precipitate during storage. If necessary, warm to 56°C until the precipitate has fully dissolved before use.

DO NOT add bleach or acidic solutions directly to the sample preparation waste. Buffers EPDL and EPDW1 contain guanidine salts, which can form highly reactive compounds when combined with bleach (sodium hypochlorite). Any spills should be cleaned with a suitable laboratory detergent and water. If the solution contains infectious agents, clean the area with detergent and water before disinfecting with 1% (v/v) bleach.

Protocol

This protocol Is designed for purification of total DNA. Multiple sample inputs are compatible with the Extracta Plus DNA kit. Extracta Plus DNA kits produce DNA yields linear over a wide range of sample input amounts from 100 cells up to $5x10^6$ cells. Larger inputs or samples with very high DNA content (i.e. spleen) may overload the capacity of the Extracta Plus Spin Column membrane. Overloading the column will produce significantly lower yields. Please see **Appendix C: Starting Sample Input Amounts (page 9)** for further guidance.

Below are two separate workflows:

(A) for purification of DNA from blood and cells from culture origin or

(B) for purification of DNA from cells from animal tissue origin.

For complete guidance regarding pre-treatment for paraffin-embedded tissue/ formalin-fixed paraffin-embedded tissue refer to **Appendix A** (page 7). For guidance regarding pre-treatment for gram-negative and gram-positive bacteria, refer to **Appendix B** (page 8).

Lysis Protocols:

(A) Total DNA from Animal Blood or Cultured Cells

Animal Blood

1. Pre-heat water bath, dry bath or heat block to 56°C.
2. Transfer 20 µl Proteinase K into a 1.5 or 2 ml microcentrifuge tube (not provided).
3. Add 50-100 µl of non-nucleated erythrocytes (mammalian) anticoagulated blood.

If non-mammalian blood is used (i.e. birds, fish or frogs), then only use 5-10 µl of anticoagulated blood.

4. Bring volume up to 220 µl with PBS.

Optional: If RNA-free genomic DNA is required, add 4 µl RNase A and incubate at room temperature for 2 min prior to proceeding with the next step.

5. Add 200 µl of Buffer EPDL. Mix by vortexing immediately and thoroughly to ensure a homogenous mixture
6. Incubate for 10 min at 56°C in pre-heated water bath, dry bath or heat block.
7. Following incubation remove from heat, add 200 µl of EtOH and immediately mix by vortexing to ensure a homogenous mixture.
   Proceed to Bind and Wash DNA steps

Cultured Cells

1. Pre-heat water bath, dry bath or heat block to 56°C.
2. Centrifuge cultured cells (up to $5x10^6$ cells) at 300 x *g* for 5 min.
3. Resuspend cells in 200 µl PBS.

   When starting with a frozen cell pellet, the pellet should be allowed to thaw before adding PBS.

4. Add 20 µl of Proteinase K, proceed to step 5.

<u>Optional:</u> If RNA-free genomic DNA is required, add 4 µl RNase A and incubate at room temperature for 2 min prior to proceeding with the next step.

5. Add 200 µl Buffer EPDL. Mix by vortexing immediately and thoroughly to ensure a homogenous mixture.
6. Incubate for 10 min at 56°C in pre-heated water bath, dry bath or heat block.
7. Following incubation remove from heat, add 200 µl of 96-100% EtOH, and immediately mix by vortexing to ensure a homogenous mixture.

Proceed to Bind and Wash DNA steps

(B) Total DNA from Animal Tissues

This protocol is suitable for up to 25 mg tissue. Certain tissues with high cellular density, such as spleen tissue, only require 10 mg input. For rodent tails, a single rat tail sample of 0.4-0.6 cm in length should be used, and for mouse tail two sections of 0.4-0.6 cm in length should be used. If using frozen tissue, equilibrate the sample to room temperature before lysis and avoid repeated freezing and thawing of the samples.

For paraffin-embedded tissue/ formalin-fixed paraffin-embedded tissue refer to Appendix A for additional pre-treatment steps before following this protocol.

1. Pre-heat water bath, dry bath or heat block to 56°C.
2. Cut up tissue into small sections to enable efficient lysis and place into a single 1.5 ml microcentrifuge tube (not provided).

Tissues sectioned into smaller pieces will lyse with higher efficiency. We recommend cutting the tissues into as small of sections as possible. Alternatively, tissues can be frozen with liquid nitrogen and ground before addition of buffer EDPTL.

3. Add 180 µl of buffer EDPTL to the tissue.
4. Add 20 µl Proteinase K to the EDPTL/tissue mix.
5. Vortex thoroughly then incubate at 56°C in pre-heated water bath, dry bath or heat block until the sample is completely lysed. Vortex occasionally during incubation to redistribute cells and allow complete lysis of tissue samples.

Complete lysis typically completes in 1-3 h. Certain tissues such as rodent tails can require 6-8 h. Lysis can be performed overnight without adverse effects.

After Proteinase K digestion, tissue samples can be stored in Buffer EDPTL at room temperature (15-25°C) for up to 6 months without any reduction in DNA quality.

<u>Optional:</u> If RNA-free genomic DNA is required, add 4 µl RNase A and incubate at room temperature for 2 min prior to proceeding with the next step.

6. Vortex for 15 s then add 200 µl of Buffer EPDL and mix by vortexing. Then add 200 uL EtOH and immediately mix by vortexing to ensure a homogenous mixture.

It is important that the lysed tissue sample, Buffer EPDL and EtOH are mixed immediately and thoroughly to give a homogenous solution. A pre-mix of Buffer EPDL and EtOH can be prepared and added to the lysed tissue in one-step to facilitate the processing of multiple samples. Pre-mixing Buffer EPDL and EtOH may result in the formation of a white precipitate that does not interfere with DNA extraction.

Proceed to Bind and Wash DNA steps

Bind and Wash DNA:

1. Assemble Extracta Plus DNA Spin Column for protocol by placing the spin column in the 2 ml collection tube provided.
2. Transfer the mixture (including any precipitate) from pre-treatment steps into the spin column and centrifuge at ≥6000 x *g* (8000 rpm) for 1 min. Place spin column on fresh collection tube and discard the used collection tube and flow-through.
3. Add 500 µl EPDW1 to the spin column. Centrifuge at ≥6000 x *g* (8000 rpm) for 1 min. Place spin column on fresh 2 ml collection tube (provided) and discard the used collection tube and flow-through.
   Add 500 µl of EPDW2 and centrifuge for 3 min at 20,000 x *g* (14,000 rpm). The extended time in this step ensures that all the EtOH from the sample is removed and in the flow through.

Take care to ensure that the spin column does not come into contact with flow-through. If any residual liquid remains in the spin column, empty flow-through and spin an additional 1 min at 20,000 x *g* (14,000 rpm).

Elute DNA:

1. Place the Extracta Plus DNA spin column in a clean 1.5 ml or 2 ml microcentrifuge tube (not provided), and pipette 200 µl of Buffer EPDE directly onto the Extracta Plus membrane. Incubate at room temperature for 1 min, then centrifuge for 1 min at ≥ 6000 x *g* (8000 rpm) to elute DNA.

DNA can be eluted in 100 µl Buffer EPDE (instead of 200 µl) to increase final DNA concentration, however, this can also decrease total DNA yield. A second elution of DNA can be carried out to recover more DNA. Use a new 1.5 ml collection tube to prevent dilution of the first eluate. A single, minimum elution volume of 50 µl Buffer EPDE (or water) is recommended for samples containing <1 µg DNA. A single elution step of 200 µl is sufficient to elute samples containing up to 10 µg DNA, but for samples containing >10 µg DNA, a second elution step with another 200 µ Buffer EPDE is recommended.

Do not exceed more than 200 µl elution into the 1.5 ml microcentrifuge tube as the collection tube will come in contact with the final sample.

Purified DNA can be stored in buffer EPDE at 4°C for up to 12 months or -20°C for >1 year.

DNA Validation and Quantification.

DNA concentration can be assessed using fluorometric (e.g. Qubit) or spectrophotometric methods (e.g. Nanodrop). DNA extracted using this kit is expected to have an A_{260}/A_{280} ratio between 1.7 and 1.9.

Quality Control

Membrane control

Membrane binding capacity was tested by determining recovery of genomic DNA.

As tested by PCR, no PCR inhibitors were released from the membrane.

Buffers

Conductivity and pH of buffers and solutions were tested and were within specifications.

Buffer EPDL was tested by preparation of genomic DNA from 200 µl of whole human blood. Average yield of genomic DNA was ≥ 90% of reference yield, with an A260/A280 ratio of 1.70 – 2.00.

Functional absence of RNase was checked for Buffer EPDW2 t .

Proteinase K

Activity was determined by incubation of hemoglobin substrate solution with Proteinase K in water, at 30°C for 10 min, followed by the determination of acid-soluble tyrosine at 750 nm.

Absence of DNase and RNase activity was checked by incubation of DNA or RNA, with Proteinase K in enzyme incubation buffer at 37°C.

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Appendix A: Sample Pre-treatment for Paraffin-Embedded Tissue / Formalin-Fixed Paraffin-Embedded Tissue

This section describes two protocols that can be used to pre-treat fixed tissue before Extracta Plus DNA extraction: paraffin removal by xylene extraction and removal of fixative by washing with PBS. This pre-treatment protocol is recommended for formalin- and alcohol-fixed, paraffin-embedded tissue. Fixatives that cause DNA crosslinking, such as osmic acid, can impact amplification performance of DNA and are not recommended.

DNA purified from paraffin-embedded tissue usually yields DNA fragments with a maximum length of 650 bp due to the fixation process. Tissue type, size and age of sample, and method of fixation can greatly impact time required for lysis and DNA yield/quality. Reduced yields are expected from fixed and paraffin-embedded tissue compared to fresh or frozen tissue, therefore elution in a lower volume of Buffer EPDE is recommended (50 – 100 µl).

Option 1: Removal of Paraffin by Extraction of DNA with Xylene

1. Pre-heat a water bath, dry bath or heat block to 37°C.
2. Place up to 25 mg paraffin-embedded tissue into a 2 ml microcentrifuge tube (not provided).
3. Add 1200 µl of xylene and vortex thoroughly.
4. Pellet the sample in a microcentrifuge for 5 min at full speed at room temperature.
5. Remove the supernatant with a pipette. Do not disturb pellet.
6. Remove residual xylene in sample by adding 1200 µl of EtOH and mixing gently by vortex.
7. Pellet sample in a microcentrifuge for 5 min at full speed at room temperature.
8. Remove the supernatant with a pipette without disturbing pellet.
9. Repeat steps 6-8 once to remove any residual xylene.
10. Incubate the open microcentrifuge tube at 37°C for 10-15 min until any remaining EtOH has evaporated.
11. Resuspend the pellet in 180 µl Buffer EDPTL.

Sample is now ready to proceed with step 4 of **Total DNA from Animal Tissues** workflow (page 4).

Option 2: Removal of Fixative by Washing with PBS

1. Place up to 25 mg of paraffin-embedded tissue into a 2 ml microcentrifuge tube (not provided).
2. Add enough PBS to cover the sample, then remove and discard the PBS.
3. Repeat step 2 once.

Sample is now ready to proceed with step 1 of **Total DNA from Animal Tissues** workflow (page 4).

Appendix B: Sample Pre-treatment for Bacteria

This section describes the pre-treatment protocols for extraction of total DNA from gram-negative bacteria such as *E. coli* or gram–positive bacteria such as *B. subtilis* and *Corynebacterium* spp. Gram-positive bacteria require additional incubation with lysozyme for cell wall lysis, as described. Ensure lysozyme lysis buffer has been prepared before beginning extraction from gram-positive bacteria.

A maximum of $2x10^9$ cells is recommended for this protocol.

Pre-treatment for Gram-Negative Bacteria

1. Pellet cells in microcentrifuge tube (not provided) by centrifuging at 5000 x *g* (7500 rpm) for 10 min.
2. Discard the supernatant.
3. Resuspend Pellet in 180 µl Buffer EDPLT.

Sample is now ready to proceed with step 4 of **Total DNA from Animal Tissues** workflow (page 4).

Pre-treatment for Gram-Positive Bacteria

Before you begin, prepare lysozyme lysis buffer:

- 20 mM Tris-HCl, pH 8.0
- 2 mM sodium EDTA
- 1.2% Triton® X-100
- Immediately before use, add lysozyme to a final concentration of 20 mg/ml

Protocol:

1. Pre-heat a water bath, dry bath or heat block to 37°C. If a secondary heating block incubator or water bath is available, set it to 56°C.
2. Pellet cells in microcentrifuge tube (not provided) by centrifuging at 5000 x *g* (7500 rpm) for 10 min.
3. Discard the supernatant.
4. Resuspend pellet in 180 µl lysozyme lysis buffer.
5. Incubate for at least 30 min at 37°C.
6. Remove from 37°C environment (set to 56°C if utilizing a single heating block incubator or water bath).
7. Add 25 µl of Proteinase K and 200 ul Buffer EDPL. Mix by vortexing.
   Do NOT pre-mix Proteinase K and Buffer EDPL. Ensure than ethanol has not been added to Buffer EDPL.
8. Incubate at 56°C for 30 min.

<u>Optional:</u> An additional incubation for pathogen inactivation at 95°C for 15 min can be added at this step if required. However, incubation at 95°C can result in some DNA degradation.

9. Add 200 µl of EtOH to the sample and mix by vortexing to obtain a homogenous solution.
   A precipitate can form during EtOH addition, which does not interfere with the Extracta Plus DNA procedure. However, it is important to collect as much of the precipitate as possible to apply to the Extracta Plus Spin Column.

Sample is now ready to proceed with step 1 of **Bind and Wash DNA** steps (page 5).

Appendix C: Starting Sample Input Amounts

Extracta Plus DNA kits produce DNA yields linear over a wide range of sample input amounts from 100 cells up to $5x10^6$ cells. Larger inputs or samples with very high DNA content (i.e. spleen) may overload the capacity of the Extracta Plus Spin Column membrane. Overloading the column will produce significantly lower yields. Table 1 gives the maximum recommended input amounts for a variety of sample types. If more than the maximum amount of starting material is used, DNA yield will be lower than expected.

Sample	Maximum recommended input	Protocol
Mammalian blood	100 µl	Total DNA from Animal Tissues (page 4) or Cultured Cells (page 3)
Bird or fish blood (with nucleated erythrocytes)	10 µl	
Cultured cells	5×10^6	
Animal tissue	25 mg	Total DNA from Animal Tissues (page 4)
Mouse tail	0.6- 1.2 cm	
Rat tail	0.6 cm	
Paraffin embedded tissue/ Formalin-fixed paraffin embedded tissue	25 mg	Appendix A: Pre-treatment (page 7)
Bacterial culture (gram negative or gram positive)	2×10^9	Appendix B: Pre-treatment (page 8)

Very small sample sizes

Extracta Plus DNA procedures are also suitable for purifying DNA from very small amounts of starting material. If the sample has less than 5 ng DNA (<10,000 copies), 3–5 µg carrier DNA (a homopolymer, such as poly-dA, poly-dT or gDNA) should be added to the starting material. Ensure that the carrier DNA does not interfere with your downstream application. To prevent any interference of the carrier with the downstream application, an RNA carrier can be used. This can be removed later by RNase digestion. DNA or RNA homopolymers can be purchased from various suppliers.