



Non-Organic DNA Extraction Kit

S4520

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

USA & Canada

Phone: +1(800) 437-7500 • Fax: +1 (951) 676-9209 • Europe +44 (0) 23 8026 2233
Australia +61 3 9839 2000 • Germany +49-6192-207300 • ISO Registered Worldwide
www.chemicon.com • custserv@chemicon.com • techserv@chemicon.com

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TABLE OF CONTENTS

I. INTRODUCTION	1
Principle of the Technique	1
II. KIT COMPONENTS	2
Storage and Handling.....	2
Precautions.....	2
Materials Required But Not Supplied	3
III. PROTOCOLS.....	5
<i>Fig. 1: Protocol Overview.....</i>	<i>5</i>
Preparation of Working Reagents	6
Sample Collection and Preparation.....	7
Bone Marrow and Whole Blood	7
<i>Fig. 2: Separation of Body Fluid/Ficoll Hypaque</i>	
<i>Solution After Centrifugation</i>	<i>8</i>
<i>Fig. 3: Separation of Whole Blood After Centrifugation</i>	<i>10</i>
Cells	11
Solid Tissues	13
Extraction Procedure	14
IV. APPENDIX	18
Troubleshooting	18
Calculations.....	18
Reference	19
Disclaimer.....	19
Warranty.....	19

I. INTRODUCTION

The Non-Organic DNA Extraction Kit is designed to extract genomic DNA from mammalian cells with high purity. Yields of 150 µg can be expected from 5×10^7 cells. This kit eliminates the need to use hazardous organic chemicals in the extraction of DNA. Should any questions arise, please contact Chemicon Technical Services at (800) 437-7500 or at techserv@chemicon.com.

Principle of the Technique

The Non-Organic DNA Extraction Kit should be used on mammalian cells from whole blood, body fluids, tissue culture, or solid tissues. The outer cell membranes are gently lysed and the nuclei are pelleted and enzymatically deproteinated. Proteins are “salted out” and spun to the bottom of the tube. Ethanol is added to the supernatant and the DNA is spooled, air dried, and resuspended. The DNA is quantified using a spectrophotometer.

II. KIT COMPONENTS

S4520 Non-Organic DNA Extraction Kit

Part Number	Description	Quantity	Storage
90365	10X Wash Solution	30 mL	Room temp.
90366	3X Suspension Buffer I	25 mL	Room temp.
90364	Lysis Buffer I	20 mL	Room temp.
90361	Protein Digesting Enzyme	25 mg	Room temp.
90363	Protein Precipitating Agent	20 mL	Room temp.
90362	Suspension Buffer II	25 mL	Room temp.
90367	DNA Isolation Loops	25 each	Room temp.

Sufficient reagents for 20 DNA Extractions of $2.5 - 5 \times 10^7$ cells or 40 DNA Extractions of $< 2.5 \times 10^7$ cells.

Storage and Handling

1. Wash Solution and Suspension Buffer I are supplied as concentrates. Dilute according to instructions under Sec. III. *Protocols, Preparation of Working Reagents*.
2. Protein Digesting Enzyme is provided as a powder. To rehydrate, see Sec. III. *Protocols, Preparation of Working Reagents*.
3. Protein Precipitating Agent is a saturated salt solution and crystals may form. Warm to 37°C - 45°C to dissolve precipitate before using.
4. Wear gloves when isolating and handling DNA to minimize the activity of endogenous nucleases. Use autoclaved pipette tips and 1.5 mL eppendorf tubes for additional protection against nucleases.

Precautions

All discarded supernatants, aspirates and cell debris should be disposed of in an appropriate biohazard container.

Materials Required But Not Supplied

Whole Blood

1. EDTA collection tubes
2. Polypropylene tubes, 15 mL and 50 mL
3. Pipettes, 10 mL
4. Hemacytometer/pipettes/reagents or automated cell counter
5. Clinical centrifuge
6. 1X PBS

Mononuclear Cells/Bone Marrow Aspirates

1. EDTA collection tubes
2. Ficoll-Hypaque solution – see Sec. IV. *Appendix*
3. Polypropylene tubes, 50 mL
4. Pipettes, 10 mL
5. Hemacytometer/pipettes/reagents or automated cell counter
6. Clinical centrifuge
7. Biohazard waste container
8. 1X PBS

Body Fluids/Tissue Culture Cells

1. Ficoll-Hypaque solution (optional) – see Sec. IV. *Appendix*
2. Polypropylene tubes, 50 mL
3. Pipettes, 10 mL
4. Hemacytometer/pipettes/reagents or automated cell counter
5. Clinical centrifuge
6. 1X PBS

Solid Tissues

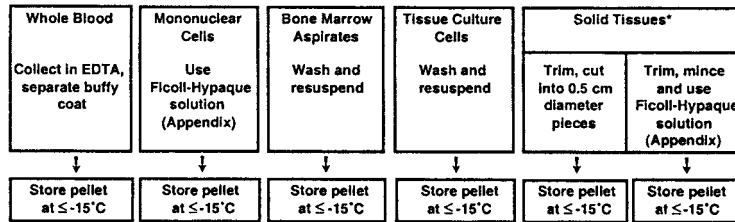
1. Scalpel, scissors, or fine wire mesh
2. Petri dish (disposable)
3. Pipettes, 10 mL
4. Polypropylene tubes, 15 mL
5. Hemacytometer/pipettes/reagents or automated cell counter
6. 1X PBS

Extraction Procedure

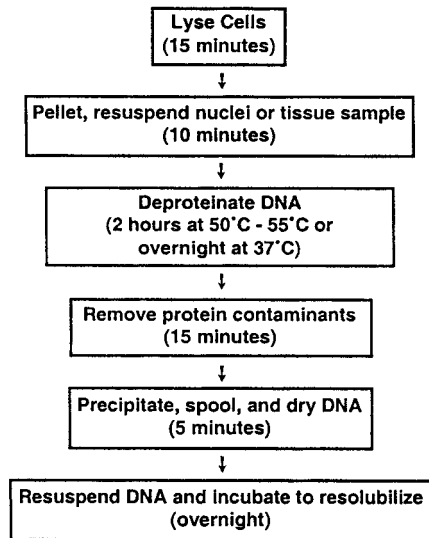
1. Deionized or distilled water
2. 70% ethanol, Absolute ethanol (100% nondenatured)
3. Polypropylene tubes, 15 mL
4. Microcentrifuge tubes, 1.5 mL
5. Pasteur pipettes
6. Micropipettor and tips, 50-1000 μ L
7. Clinical centrifuge
8. Microcentrifuge
9. Vortex
10. Spectrophotometer/Quartz cuvettes (260/280 nm)
11. 50°C \pm 2°C water bath

III. PROTOCOLS

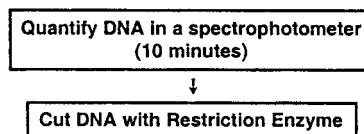
Figure 1. Protocol Overview.
SAMPLE COLLECTION AND PREPARATION



DNA EXTRACTION FLOW CHART



EXTRACTION COMPLETE



*NOTE: Although nuclei are not always generated when isolating DNA from tissue samples, we recommend following the identical protocol. The use of Ficoll-Hypaque solution is optional when extracting lymph node or spleen tissue.

Preparation of Working Reagents

1X Wash Solution

To prepare add:

2 mL	10X Wash Solution
18 mL	dH ₂ O
<hr/>	
20 mL	Total

Mix thoroughly. Diluted Wash Solution may be stored at 2°C to 8°C for 30 days.

1X Suspension Buffer I

To prepare add:

5 mL	3X Suspension Buffer I
10 mL	dH ₂ O
<hr/>	
15 mL	Total

Mix thoroughly. Diluted Suspension Buffer I may be stored at 18°C to 25°C for 30 days.

Protein Digesting Enzyme

Remove metal cap from bottle of Protein Digesting Enzyme. Carefully remove the rubber stopper; powder may be clinging to the stopper.

To prepare add 1.25 mL of sterile distilled water to the bottle and replace the rubber stopper. Invert the bottle several times to dissolve.

Keep reconstituted Protein Digesting Enzyme on ice during use and store long term at -15°C to -25°C. Aliquoting is not necessary. Final concentration is 20 mg/mL.

Preparation of Ficoll-Hypaque solution

Combine in a graduated glass beaker:

9.4 g	Sodium diatrizoate (MW 653.9)
6.3 g	Ficoll Type 400 (MW 400,000)

Add 70 mL of distilled or deionized water. Dissolve using a magnetic stirrer. Add distilled or deionized water to a final volume of 100 mL. Filter sterilize through 0.45 micron filter. Store in a sterile glass bottle. Label as "Ficoll-Hypaque" with the date of preparation and an expiration date of six months. Store at 18°C to 25°C.

Sample Collection and Preparation

Mammalian whole blood, mononuclear cells, body fluid cell suspensions/tissue culture cells, bone marrow aspirates and solid tissues can all serve as samples for this kit.

For most Southern analyses, 10 µg of DNA is required for each restriction enzyme digest. Many probe-based assays require several separate enzyme digestions and/or multiple probing of a single digestion. To ensure sufficient DNA for Southern analysis, each DNA extraction should yield 75–140 µg of DNA.

To obtain 75–140 µg of DNA, a minimum of 2.5×10^7 cells is required. A single diploid human cell contains approximately 6×10^{-12} grams of DNA; therefore, 2.5×10^7 cells contains approximately 150 µg of DNA. Assuming that the DNA extraction procedure yields 50–95% of the available DNA in the cell, a yield of 75–140 µg of DNA can be expected.

Bone Marrow and Whole Blood

Bone Marrow

Collect in EDTA. Do not use heparin as an anticoagulant. Minimum sample size is 0.5 mL; optimum is 1.0 mL. Bone marrow may be stored at 2°C to 25°C for at least 24 hours prior to use. For longer term storage, isolate cells by MONONUCLEAR CELL ISOLATION or BUFFY COAT ISOLATION as described below.

Whole Blood

Collect in EDTA. Using heparin as an anticoagulant will reduce the DNA yield. Whole blood may be stored at 2°C to 25°C for up to 24 hours prior to use. For longer term storage, isolate cells by MONONUCLEAR CELL ISOLATION or BUFFY COAT ISOLATION as described below.

1. For specimens containing $> 12,000$ WBC/mm³, collect 5-10 mL and proceed to MONONUCLEAR CELL ISOLATION.
2. For specimens containing 5,000 - 12,000 WBC/mm³ with suspected granulocytosis, collect 10-15 mL and proceed to MONONUCLEAR CELL ISOLATION.

- For specimens containing 5,000 - 12,000 WBC/mm³, collect 5-10 mL and proceed to BUFFY COAT ISOLATION.
- For specimens containing < 5,000 WBC/mm³, collect more blood following the guidelines in Table 1 and proceed to BUFFY COAT ISOLATION.

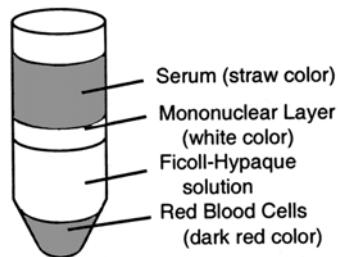
Table 1: Blood Volume Required

WBC/mm ³	Volume
$4 \times 10^3 - 5 \times 10^3$	10-20 mL
$3 \times 10^3 - 4 \times 10^3$	10-20 mL
$2 \times 10^3 - 3 \times 10^3$	20-30 mL
$1 \times 10^3 - 2 \times 10^3$	40 mL
$5 \times 10^2 - 1 \times 10^3$	40 mL

Mononuclear Cell Isolation

- Transfer body fluid (5 mL or less) to a 15 mL polystyrene tube. Transfer body fluid (6-15 mL) to a 50 mL tube. Use as many tubes as needed.
- Add an equal volume of 1X PBS to the specimen and mix well by inverting.
- Carefully underlayer with 1/3 volume of Ficoll-Hypaque solution (see Sec. III. *Protocols, Preparation of Working Reagents* for preparation).
- Centrifuge at $400 \times g$ for 30 minutes at 18°C to 25°C.

Figure 2. Separation of Body Fluid/Ficoll-Hypaque Solution after Centrifugation.



- Aspirate off the clear plasma layer to 2-3 mm above the top of the mononuclear layer and discard into a biohazardous waste beaker.
- Remove the mononuclear cell layer plus about half of the Ficoll-Hypaque layer below it with a pipette and transfer to a new polypropylene tube of the same volume as used in Step 1. Avoid red blood cells at the bottom of the tube.

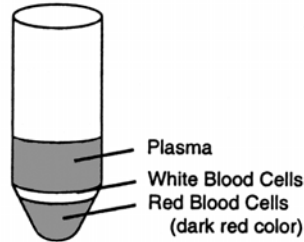
7. Fill the tube with 1X PBS.
8. Centrifuge at $400 \times g$ for 10 minutes at 18°C to 25°C .
9. Decant supernatant into biohazardous waste beaker and repeat wash described in steps 7 and 8.
10. Pour off supernatant and resuspend cells in 10 mL of 1X PBS.
11. Remove 0.5 mL of cell suspension to 15 mL tube to count.
12. Add 4.5 mL of 1X PBS to the 0.5 mL of cell suspension. Mix well by inversion or vortex gently.
13. Fill hemacytometer with cell suspension.
14. Count and record the clear cells found in one large center square under a light microscope. (One large square = 25 small squares.)
15. Calculate the cell concentration and the total # of cells in the sample.
Clear cells $\times 10$ (dilution factor) $\times 10^4 = \# \text{ cells/mL}$
Cells/ml $\times 10 \text{ ml (total volume)} = \text{Total \# cells}$
16. Proceed by comparing total # of cells in sample to number of cells in Table 2 and following specified action.
17. In all cases, the sample may be frozen at this step prior to extraction.
 - a) Centrifuge the tube containing the sample at $400 \times g$ for 5 minutes at 18°C to 25°C . Pour the supernatant fraction into a biohazardous waste beaker. Freeze the tube containing the cell pellet at -15°C to -25°C for up to two weeks or at $< -70^{\circ}\text{C}$ for one year or longer.
 - b) To proceed to the extraction protocol, centrifuge the tube containing the sample at $400 \times g$ for 5 minutes at 18°C to 25°C . Pour off supernatant and proceed to EXTRACTION PROCEDURE.

Buffy Coat Isolation

1. Place blood collected in EDTA in a 50 mL polystyrene tube.
2. Centrifuge at $400 \times g$ for 20 minutes at 18°C to 25°C .

Figure 3. Separation of Whole Blood after Centrifugation.

3. Carefully collect plasma (clear fluid) using a 10 mL pipet, leaving approximately 3 mm layer above white blood cells. Do not disturb the thin layer of white blood cells.



4. Discard plasma into biohazardous waste container.
5. Using the same 10 mL pipet, collect the layer of white blood cells (buffy coat) and a small amount of red blood cells and place in a new 15 mL polypropylene centrifuge tube.
6. Add an equal amount of 1X PBS to the 15 mL centrifuge tube containing white blood cells; mix gently by inversion.
7. Centrifuge at $400 \times g$ for 5 minutes at 18°C to 25°C .
8. Using a 5 mL pipet, remove top clear layer (wash solution/plasma) and discard into biohazardous waste container.
9. Remove white blood cell layer carefully, without taking up red blood cells, and place in a new 15 mL polypropylene tube.
10. Add 1X PBS to a total volume of 10 mL. Invert to mix.
11. Remove 0.5 mL of cell suspension (sample to be extracted) to a new 15 mL tube.
12. Add 4.5 mL of 1X PBS to 0.5 mL of cell suspension sample (1:10 dilution). Mix well by inversion or vortex gently.
13. Load approximately 100 μL of cell suspension onto a hemacytometer.

14. Count and record the clear cells found in one large center square under a light microscope. (One large square = 25 small squares.)
15. Calculate the cell concentration and the total # of cells in the sample as described in step 15 of MONONUCLEAR CELL ISOLATION.
16. Proceed by comparing total # of cells in sample to number of cells in Table 3 and following specified action.
17. Refer to MONONUCLEAR CELL ISOLATION step 17A for storing the sample or MONONUCLEAR CELL ISOLATION step 17B for proceeding to the EXTRACTION PROCEDURE.

Table 2: Protocol Steps Corresponding to Cell Number

Tot l # of cells in millions	Action
<25	Centrifuge at 1000 × g for 5 minutes. Discard supernatant and resuspend cells in 5 mL of 1X PBS. Perform extraction using one half of all volumes of reagents mentioned.
25–100	Proceed to extraction.
>100	Mix sample by inversion and remove 5 mL to a new polypropylene tube. Centrifuge new tube at 400 × g for 10 minutes at 18°C to 25°C. Decant supernatant into biohazardous waste beaker and freeze the tube containing cell pellet at < -15°C. Dilute sample to final volume of 10 mL with 1X PBS and proceed to extraction with the other half of the sample.

Cells

Use body fluids containing cells in suspension, cell pellets from MONONUCLEAR CELL ISOLATION or BUFFY COAT ISOLATION, or tissue culture cells. Sample size is 1×10^6 Cells – 2.5×10^7 cells.

Cell Pellets

Store at -15°C to -25°C for up to two weeks, $\leq -70^\circ\text{C}$ for one year or longer, or proceed directly to EXTRACTION PROCEDURE.

Tissue Culture Cells or Cells in Suspension

Fresh Samples:

1. Collect 1×10^6 – 2.5×10^7 cells in a 15 mL conical polypropylene tube.
2. Centrifuge cells at $400 \times g$ for 5 minutes at room temp (18°C to 25°C).
3. Decant supernatant into a beaker for biohazardous waste.
4. Suspend cells in 10 mL of 1X PBS.
5. Centrifuge cells at $400 \times g$ for 5 minutes at room temp (18°C to 25°C).
6. Decant supernatant into a beaker for biohazardous waste.
7. Proceed to EXTRACTION PROCEDURE. The cell pellet may be stored at -15°C to -25°C for up to two weeks, or at $\leq -70^\circ\text{C}$ for one year or longer.

Ethanol-Fixed Samples:

1. Collect 1×10^6 – 2.5×10^7 cells in a 15 mL conical polypropylene tube.
2. Centrifuge cells at $400 \times g$ for 5 minutes at room temp (18°C to 25°C).
3. Decant supernatant into a beaker for biohazardous waste.
4. Suspend cells in 5 mL of 1X PBS.
5. Add 5 mL of 100% ethanol to the 5 mL cell suspension.
6. Cap tightly and invert 5 times to mix.
7. Store the sample at room temp (18°C to 25°C) for up to two weeks prior to use or proceed to Ethanol Removal Procedure.

Ethanol Removal Procedure:

1. Transfer cell suspension to a 50 mL conical tube.
2. Centrifuge at $400 \times g$ for 5 minutes at room temp (18°C to 25°C).
3. Pour off supernatant and add 40 mL of 1X PBS.
4. Incubate samples in 1X PBS for 15 minutes at room temp (18°C to 25°C) with occasional gentle shaking.
5. Centrifuge at $400 \times g$ for 5 minutes at room temp (18°C to 25°C).
6. Pour off supernatant, add 40 mL of 1X PBS and repeat steps 4 and 5.
7. Pour off supernatant, add 40 mL of 1X PBS and incubate for 1–24 hours in a refrigerator (2°C to 8°C).

8. Centrifuge at $400 \times g$ for 5 minutes at room temp (18°C to 25°C).
9. Pour off supernatant and freeze pellet at -15°C to -25°C for up to two weeks, $\leq -70^{\circ}\text{C}$ for one year or longer, or proceed directly to EXTRACTION PROCEDURE.

Solid Tissues

Solid tissue may be fresh, frozen un-embedded, frozen OCT-embedded, or ethanol fixed. Optimum sample size is a cube 3–5 mm on each side (**25–125 mg wet weight**).

Fresh, Frozen, or OCT-Embedded Samples

1. Place biopsy in a 100×15 mm polystyrene petri dish. For OCT-embedded tissue, cut desired size immediately; OCT will melt as it warms. Return unused portion to -15°C to -25°C for up to two weeks or $\leq -70^{\circ}\text{C}$ for one year or longer. Allow the OCT surrounding the tissue in the petri dish to melt. Remove tissue from OCT to another petri dish.
2. Cover tissue with 0.5 – 1 mL 1X PBS.
3. Cut away fat and connective tissue with scalpel and discard.
4. Cut tissue to recommended size with scalpel; freeze unused portion at -15°C to -25°C for up to two weeks, or at $\leq -70^{\circ}\text{C}$ for one year+.
5. Mince tissue finely with a scalpel.
6. Transfer minced tissue in 1X PBS to a 15 mL polypropylene conical tube.
7. Proceed to EXTRACTION PROCEDURE.

Ethanol Fixed Samples

Note: Do not use this procedure on OCT-embedded samples.

1. Collect fresh or frozen biopsy in a 15 mL conical, polypropylene tube.
2. Add 5 mL of 1X PBS to the tube.
3. Add 5 mL of 100% ethanol to the tube.
4. Cap tightly and invert 5 times to mix.
5. Store at room temperature (18°C to 25°C) for up to 2 weeks prior to use.
6. Proceed to Ethanol Removal Procedure.

Ethanol Removal Procedure

1. Remove tissue from ethanol and place in a petri dish.
2. Cut off a piece which is 2 mm³ – 3 mm³ in size and mince finely with scissors or razor blade.
3. Transfer to 50 mL tube and add 40 mL of 1X PBS. Remaining Sample: Cut into sample size pieces and transfer to a 50 mL tube. Fill tube with 1X PBS, and let it equilibrate in the refrigerator (2°C to 8°C) overnight, changing the buffer 3 times during the day. Next day, pour off the 1X PBS, label tube with the appropriate identification information, and store at or below -15°C to -25°C for up to two weeks or at ≤ -70°C for one year or longer.
4. Incubate samples in 1X PBS for 15 minutes at room temperature (18°C to 25°C) with occasional gentle shaking.
5. Centrifuge at 400 × g for 5 minutes at room temp. (18°C to 25°C).
6. Pour off supernatant, add 40 mL of 1X PBS, and repeat steps 4 and 5.
7. Pour off supernatant, add 40 mL of 1X PBS and incubate for 1–24 hours in a refrigerator (2°C to 8°C).
8. Centrifuge at 400 × g for 5 minutes at room temp. (18°C to 25°C).
9. Pour off supernatant and freeze pellet at -15°C to -25°C for up to two weeks, ≤ -70°C for one year or longer, or proceed directly to EXTRACTION PROCEDURE.

Note: Estimate the volume of liquid waste from specimen preparation. Use an appropriately sized glass beaker. Add full strength bleach equivalent to 10% of beaker's total volume.

Extraction Procedure

Note: Gloves must be worn when performing this procedure.

1. Add 1X Wash Solution to a final volume of 9 mL. Invert to mix. Incubate 15 minutes at room temp.
2. Centrifuge at 1000 × g for 20 minutes at 18°C to 25°C. Discard supernatant fluid.

Note: For optimum DNA yields, change the volumes for steps 3-6 of the EXTRACTION PROCEDURE according to Table 3.

Table 3: Volume Adjustments Based on Cell Number

Cell Count	Adjustment to Volumes Provided
$<2.5 \times 10^7$	0.5X
$2.5 \times 10^7 - 5 \times 10^7$	no change
$5.0 \times 10^7 - 7.5 \times 10^7$	1.5X
$7.5 \times 10^7 - 10.0 \times 10^7$	2.0X

3. Suspend the nuclei pellet in 3 mL of 1X Suspension Buffer I using a pipette to aspirate and disperse clumps.
4. Add 800 μ L Lysis Buffer I and vortex 10 seconds to mix. The solution will become viscous.
5. Add 50 μ L of Protein Digesting Enzyme. Invert to mix and incubate overnight at 37°C or 2 hours at 50°C to 55°C.
6. To each sample, add 1 mL Protein Precipitating Agent and shake vigorously for 15 seconds. The sample should foam.
7. Centrifuge at 1000 \times g for 15 minutes at 18°C to 25°C.
8. Pipet off and save supernatant in a clean 15 mL tube. Discard pellet (which may not be visible).
9. To the supernatant, add two volumes of room temperature (18°C to 25°C) absolute ethanol. It is very important **not** to add cold ethanol, which will result in an insoluble precipitate of salts, DNA, and remaining protein.
10. Invert gently 10–15 times until solution is homogeneous and DNA precipitates. DNA will appear as a white floccule.
11. Retrieve DNA by spooling with a DNA Isolation Loop. Set the loop on a rack and allow the DNA to air dry for 4 to 5 minutes.
12. Carefully dip the loop with the DNA into a 15 mL tube containing 5 mL 70% ethanol, and swirl gently. Reset the loop on a rack and allow the DNA to air dry for 4 to 5 minutes.

13. Refer to the previously recorded cell count and Table 4 to determine the volume of Suspension Buffer II to add at this step.

Table 4: Volume of Suspension Buffer II Required

Cell Count	Volume
$<2.5 \times 10^7$	100 μL
2.5×10^7	150 μL
5.0×10^7 (or 3 – 5 mm^3 placenta)	300 μL
$7.5 \times 10^7 - 10.0 \times 10^7$	450 μL

Add the required volume of Suspension Buffer II to a 1.5 mL microcentrifuge tube. Place the loop with the DNA into the solution and rotate (spin) the loop between thumb and forefinger. The DNA will appear as a mass that settles to the bottom of the tube. Vortex for 5–10 seconds (if the DNA does not come off the loop, break off the loop end in the tube and leave it there until the DNA is solubilized).

14. Place the tube with DNA in a 50°C water bath overnight.
15. Determine the amount of DNA in solution:
- For samples in more than 200 μL of Suspension Buffer II, dilute an aliquot of sample 1:20 by adding 50 μL of DNA solution to 950 μL of deionized or distilled water in a 1.5 mL microcentrifuge tube. Vortex dilution and transfer to an appropriate cuvette. Read and record the A_{260} and A_{280} of the 1:20 dilution.
 - For samples in 200 μL or less of Suspension Buffer II, dilute an aliquot of sample 1:50 by adding 20 μL of DNA solution to 980 μL of distilled water in a 1.5 mL microcentrifuge tube. Vortex dilution and transfer to an appropriate cuvette. Read and record the A_{260} and A_{280} of the 1:50 dilution.
 - Calculate the DNA concentration according to Sec.IV. *Appendix, Calculations.*

16. Calculate A_{260}/A_{280} ratio; if less than 1.6, first check to make sure DNA is in solution. DNA should pipet as easily from the center of the tube as from the sides (add Suspension Buffer II in 50 μL increments until DNA pipets easily after heating in a 50°C water bath). If DNA is completely in solution and the A_{260}/A_{280} ratio is still less than 1.6, the sample must be re-extracted. Dilute the DNA up to 1.5 mL with Suspension Buffer I and continue from Step 4 of the Extraction Procedure with 1/2 stated volumes.

Note: If the A_{260}/A_{280} ratio is still less than 1.6, continue with the procedure. DO NOT re-extract again.

17. Determine the DNA concentration in $\mu\text{g}/\mu\text{L}$ and total yield in mg according to Sec. IV. *Appendix, Calculations.*
18. If $\mu\text{g}/\mu\text{L}$ is less than 0.244, precipitate the sample as follows. For every 1.0 mL of resuspended DNA, add:
 - a. 260 μL of Protein Precipitating Agent and mix.
 - b. 2.52 mL of room temperature absolute ethanol.
 - c. Proceed to steps 10–18.
19. Proceed to Digestion or store DNA in one of three ways:
 - a. 2°C to 8°C for up to one month.
 - b. -15°C to -25°C for up to 6 months.
 - c. $\leq -70^\circ\text{C}$ indefinitely.

IV. APPENDIX

Troubleshooting

1. DNA that is degraded may be the result of a mishandled tissue sample. Tissue that is held at room temperature can result in degraded DNA. Care should be taken that all starting samples, including cell pellets, are handled on ice.
2. Whole blood should not be stored for more than 24 hours before being processed. After performing a buffy coat isolation, the white blood cells can be stored at $\leq -70^{\circ}\text{C}$ for up to 1 year.
3. When resuspending the DNA in Suspension Buffer II, do not heat the DNA higher than 55°C . This can cause degradation of the DNA.
4. Spermidine may be added to the Protein Digestion Enzyme mixture at a final concentration of 4 mM to aid in the protection of the genomic DNA.

Calculations

Determination of DNA concentration ($\mu\text{g}/\mu\text{L}$):

$$A_{260} \times \text{dilution factor} \times \frac{50 \mu\text{g}}{1 \text{ mL}} \text{ of DNA per absorbance unit} \times \frac{1 \text{ mL}}{1000 \mu\text{L}} = \frac{\mu\text{g}}{\mu\text{L}}$$

$$\text{Therefore, } A_{260} \times \frac{\text{dilution factor}}{20} = \frac{\mu\text{g DNA}}{\mu\text{L}}$$

Where dilution factor = 20 or 50, this calculation reduces to:

$$A_{260} \mu\text{g DNA}/\mu\text{L for 1:20} \quad \text{OR} \quad A_{260} \times \frac{50}{20} = \mu\text{g DNA}/\mu\text{L for 1:50}$$

Example 1. Made a 1:20 dilution of sample #1;
 $A_{260} = 0.700$ (DNA) = $0.700 \mu\text{g}/\mu\text{L}$

Example 2. Made a 1:50 dilution of sample #2;
 $A_{260} = 0.700$ (DNA) = $\frac{0.7 \times 50}{20} = 1.75 \mu\text{g}/\mu\text{L}$

Determination of DNA total yield in µg:

Total volume of DNA suspension × µg/µL = total µg

Example: 400 µL × 0.700 µg/µL = 280 µg

Reference

1. Dykes, D. (1988) “The use of biotinylated DNA probes in parentage testing: non-isotopic labeling and non-toxic extraction.” *Electrophoresis* 9: 359-368.

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Warranty

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