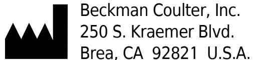
Instructions For Use

GenFind V3

Blood, Cell, & Serum
Genomic DNA Isolation Kit

C36038AB
May 2019



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

GenFind V3
Blood, Cell, & Serum Genomic DNA Isolation Kit
PN C36038AB (May 2019)

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

Product Availability

REF C34880 — GenFind V3 Small Kit
200 µL Samples – 50 preps; 400 µL Samples – 25 preps

REF C34881 — GenFind V3 Large Kit
200 µL Samples – 384 preps; 400 µL Samples – 192 preps

REF C42216 — GenFind V3 4800 Prep Kit
200 µL Samples – 4800 preps; 400 µL Samples – 2400 preps

Find us on the World Wide Web at:
www.beckman.com

Beckman Coulter Eurocenter S.A.
22, rue Juste-Oliver
Case Postale 1044
CH - 1260 Nyon 1, Switzerland
Tel: +41 (0) 22 365 36 11

Revision History

This document applies to the latest version and higher versions. When a subsequent version changes the information in this document, a new issue will be released to the Beckman Coulter website. For updates, go to www.beckman.com/techdocs and download the latest version of the manual.

Revision AA, 12/2018
Initial Release

Revision AB, 05/2019
Updated the following sections for the addition of the 4800 Prep Kit:

- *Product Availability*
- *Kit Specifications*
- *Materials Supplied*
- *Sample Preparation*

Protocol for GenFind V3

Blood, Cell, & Serum Genomic DNA Isolation Kit

GenFind V3 is for molecular biology research use only. Not for use in diagnostic procedures.

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Product Description

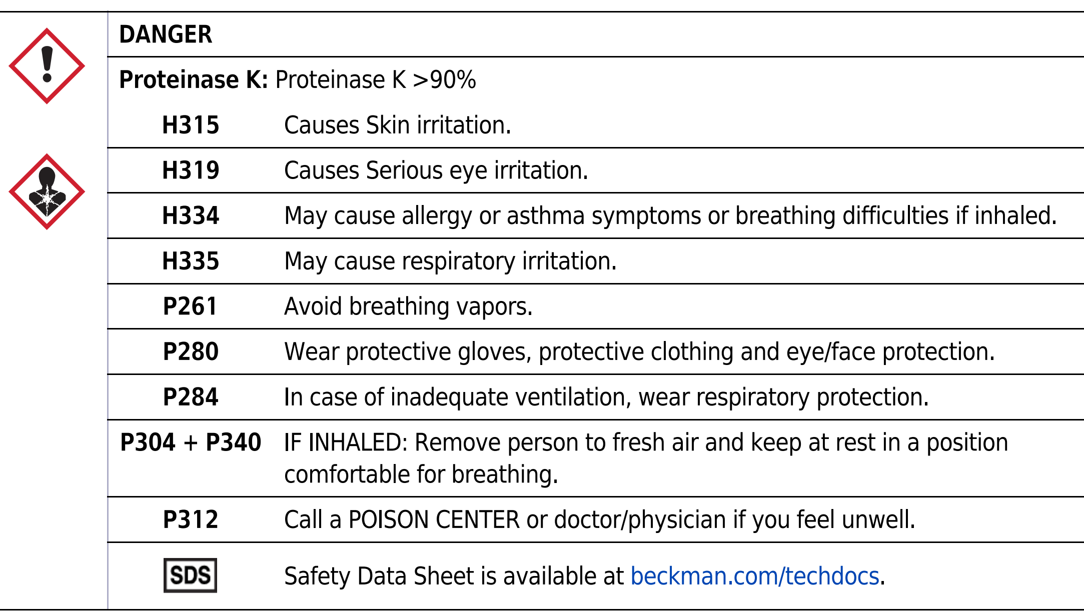
The GenFind V3 Blood, Cell, & Serum DNA Isolation Kit is used for isolating genomic DNA from fresh or frozen whole blood and serum containing Citrate, EDTA, or Heparin anticoagulants, as well as cultured cells. This kit is designed for input volume range from **50 µL** to **400 µL**. The protocol can be performed in both 96-well plate and single tube formats.

Kit Specifications

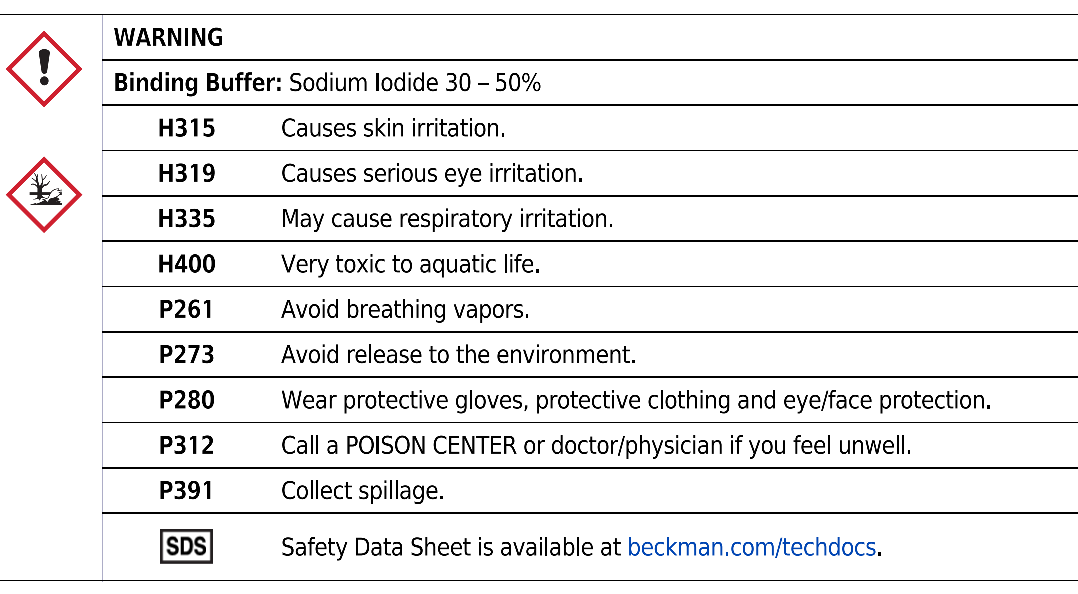
Sample Size	Small Kit (C34880)	Large Kit (C34881)	4800 Prep Kit (C42216)
200 µL Sample	50 preps	384 preps	4800 preps
400 µL Sample	25 preps	192 preps	2400 preps

Statement of Warnings

1. Specimens, samples, and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
2. Do not use reagent beyond the expiration date on the vial label.
3. Use Good Laboratory Practices (GLP) when handling reagent.



DANGER	
Proteinase K: Proteinase K >90%	
H315	Causes Skin irritation.
H319	Causes Serious eye irritation.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335	May cause respiratory irritation.
P261	Avoid breathing vapors.
P280	Wear protective gloves, protective clothing and eye/face protection.
P284	In case of inadequate ventilation, wear respiratory protection.
P304 + P340	IF INHALED: Remove person to fresh air and keep at rest in a position comfortable for breathing.
P312	Call a POISON CENTER or doctor/physician if you feel unwell.
SDS	Safety Data Sheet is available at beckman.com/techdocs.



WARNING	
Binding Buffer: Sodium Iodide 30 – 50%	
H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.
H400	Very toxic to aquatic life.
P261	Avoid breathing vapors.
P273	Avoid release to the environment.
P280	Wear protective gloves, protective clothing and eye/face protection.
P312	Call a POISON CENTER or doctor/physician if you feel unwell.
P391	Collect spillage.
SDS	Safety Data Sheet is available at beckman.com/techdocs.

	WARNING
Lysis Buffer: Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate 1 – 5%	
Polyoxyethylated Octyl Phenol <3%	
H316	Causes mild skin irritation.
H319	Causes serious eye irritation.
P280	Wear protective gloves, protective clothing and eye/face protection.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P332+P313	If skin irritation occurs: Get medical advice/attention.
P337+P313	If eye irritation persists: Get medical advice/attention.
SDS	Safety Data Sheet is available at beckman.com/techdocs.

Storage and Stability

NOTE Refer to the product labels for expiration dates.

Reagent	Storage Condition
Lysis LBB	Room Temperature
Proteinase K	Room Temperature
Bind BBB	4°C DO NOT FREEZE
Wash WBB	Room Temperature DO NOT REFRIGERATE, DO NOT HEAT if white precipitate is present, shake gently to resuspend
Wash WBC	Room Temperature; add Ethanol before use

Materials Supplied

Small Kit (C34880)	Large Kit (C34881)	4800 Preps Kit (C42216)	Reagent	Icon
REF C34816	REF C34822	REF C42161	Lysis LBB	LBB
REF C34821	REF C34827	REF C43773	Proteinase K	–
REF C34817	REF C34823	REF C42162	Bind BBB	BBB
REF C34819	REF C34825	REF C42164	Wash WBB	WBB
REF C34820	REF C34826	REF C42165	Wash WBC	WBC

Materials Required but not Supplied

Consumables and Hardware

Item	Type
Magnetic Separator	**For 96 well format:** V&P Bar Magnetic Separation Plate (V&P product: VP-771MWZM-1-ALT)
	For Tube Format: SPRIStand Magnetic 6 Tube Stand (Beckman Coulter product #A29182, www.beckman.com)
Reaction Plate	**For 96 well Plate Format: Sterile SQ Well Plate** (Beckman Coulter product #609681, www.beckman.com)

Reagents

<table>
<tr><th colspan="2">Item</th><th>Supplier
Catalog Number</th></tr>
<tr><td colspan="2">100% Ethanol</td><td></td></tr>
<tr><td colspan="2">PBS (Phosphate-Buffered Saline)

NOTE only required for small volume extractions and/or cell cultures</td><td>Thermo Fisher Scientific
10010023 or
equivalent</td></tr>
<tr><td rowspan="3">Elution Buffer</td><td>TE Buffer
10 mM Tris-HCl, 1 mM EDTA, pH 8.0
OR</td><td>Fisher Scientific
BP24731, or equivalent</td></tr>
<tr><td>Tris HCl, 1M Solution, pH 8.0
OR</td><td>American Bioanalytical
AB14043, or equivalent</td></tr>
<tr><td>Reagent Grade Water</td><td>Ambion
AM9932, or equivalent</td></tr>
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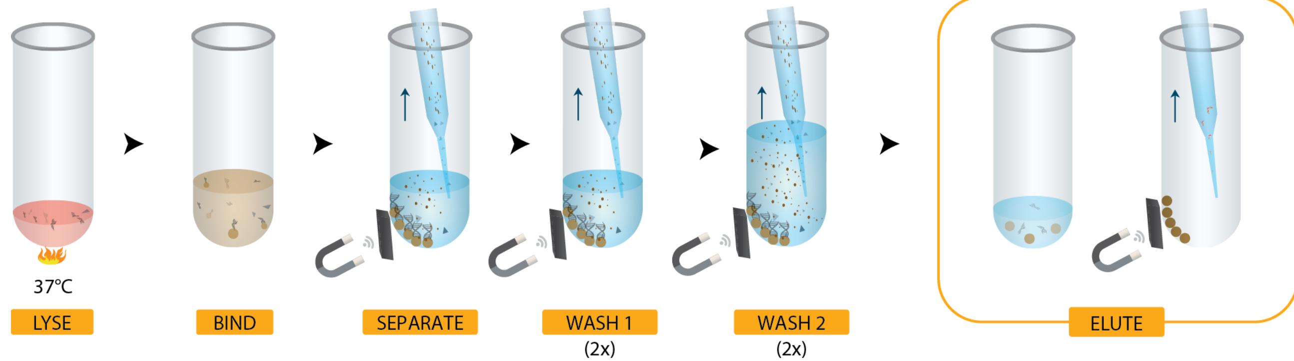
Process Overview



1. Lyse whole blood or serum in **Lysis** (LBB) and **Proteinase K.**
2. Bind genomic DNA with **Bind** (BBB) to paramagnetic beads.
3. Separate beads from contaminants.
4. Wash the magnetic beads twice with **Wash** (WBB) to remove contaminants.
5. Wash the magnetic beads twice with **Wash** (WBC) to remove contaminants.
6. Elute DNA from magnetic particles.
7. Transfer to new plate.

Sample Preparation

1 For each new test kit, assemble **Wash** (WBC) according to the instructions listed on the bottle.

Volume of 100% ethanol to add to each bottle of Wash WBC concentrate	
Small Kit (C34880)	72 mL
Large Kit (C34881)	576 mL
4800 Prep Kit (C42216)	750 mL

2 **Starting Material:** GenFind V3 Blood, Cell, & Serum Kit can be used with fresh or frozen whole blood containing Citrate, EDTA, or Heparin anticoagulants, as well as cultured cells.

a. Thaw frozen samples at **room temperature** or **37°C**.

b. Mix the blood or serum by gently inverting the stock tube several times. Tip mixing or vortexing is not recommended.

c. Resuspend cultured cells (up to 2 million cells) in **200 µL PBS**.

d. Aliquot **200 µL** of fresh or frozen blood, or up to 2 million cells into the magnet compatible **2 mL** 96-well plate.

NOTE Mixing blood thoroughly before aliquoting helps to increase yield. For a plate to be 'magnet compatible', the bottom of each well should directly contact the magnet. Ideally, the wells should have a round bottom without any plastic extrusions.

3 For sample volumes of **200 to 400 µL**, go to step 1, below.

a. For sample volumes less than **200 µL**, add **PBS** to the sample to increase the volume to **200 µL** (The sample volume now will be **200 µL** for the calculation in step 1, below).

NOTE Using a working volume lower than **200 µL** can result in decreased yields.

Procedure (For up to 400 µL of Blood/Serum)

The 96 well plate format allows purification up to **400 µL** of blood/cells/serum per well. The protocol below lists reagent additions based on a **200 µL** starting volume. The reagent volumes should be scaled linearly when directed if starting with different sample volumes.

NOTE Beckman Coulter, Inc. strongly recommends using aerosol-barrier (filter) pipette tips when performing the GenFind V3 purification.

1 Add **Lysis** LBB.

a. To determine volume, calculate: 2 × sample volume + 100 µL = volume of **Lysis LBB**.

Example: 2 × 200 µL sample volume + 100 µL = 500 µL Lysis

.

Example Sample Volume	Volume of Lysis LBB
200 µL	500 µL
400 µL	900 µL

2 Add **Proteinase K**.

a. To determine volume, calculate 0.15 × sample volume.
Example: 0.15 × 200 µL sample volume = 30 µL **Proteinase K**

Example Sample Volume	Volume of Proteinase K
200 µL	30 µL
400 µL	60 µL

b. Gently pipette tip mix 10 times or until well mixed.

c. When lysing the samples, use a mix volume that is slightly less than the total volume in the well and pipette slowly to minimize the formation of air bubbles. Bubbles can decrease overall yield and purity.

3 Incubate the samples at **37°C** for **10 minutes**.

a. For lysis at **37°C**, samples can be sealed and placed in an incubator. For sample volume larger than 200 µL, incubate the plate for additional 5 minutes for better lysis.

b. **Room temperature** incubation for **30 minutes** can also be utilized if heated incubation is not available. *Yield results may vary with room temperature incubation compared to heated lysis.*

IMPORTANT Vortex or invert the **Bind** BBB bottle at least 20 times to ensure complete resuspension of magnetic particles before using.

4 Add **Bind** BBB.

a. To determine volume, calculate 1.5 × sample volume.

Example: 1.5 × 200 µL sample volume = 300 µL **Bind** .

Example Sample Volume	Volume of Bind BBB
200 µL	300 µL
400 µL	600 µL

b. Gently pipette tip mix 10 times or until well mixed.

NOTE During this step, DNA binds to the magnetic particles. When mixing, use a mix volume that is slightly less than the total volume in the well and pipette slowly to minimize the formation of air bubbles. Air bubbles can trap magnetic beads and prevent them from being pulled to the bottom of the plate, thus decreasing yield.

5 Incubate the plate at **room temperature** for **5 minutes** to bind.

6 Place the sample plate on the magnet for **15 minutes** to separate.

a. The solution will be very dark in color and it will be difficult to see the beads form a pellet at the bottom of the plate. As long as the samples have been allowed to separate for the specified time, it can be assumed that a complete bead pellet has formed.

b. For sample volume larger than 200 µL, leave the sample plate on the magnet for additional 5 minutes for better separation.

7 Aspirate off the supernatant and discard while the plate is situated on the magnet.

a. Due to the large volume of supernatant, this step may require multiple aspirations to remove all the liquid. It will be difficult to see the beads at the bottom of the well until the liquid level gets low. When aspirating, place the pipette to the opposite side of the bar magnet and follow the liquid level to avoid disturbing the magnetic beads.

8 Take the plate off the magnet.

a. Add **Wash** WBB.

To determine volume, calculate 4 × sample volume.

Example: 4 × 200 µL sample volume = 800 µL **Wash** .

Example Sample Volume	Volume of Wash WBB
200 µL	800 µL
400 µL	1600 µL

b. Pipette tip mix 10 times (with a 1 mL pipette set to 700 µL or until the magnetic beads are resuspended from the bottom of the well. A few beads may still stick to the bottom of the well, and some of the resuspended beads may form clumps. For 400 µL samples, adding 800 µL of wash buffer and resuspending before adding the remaining 800 µL of the wash buffer, may make bead resuspension easier.

9 Place the plate back on the magnet for **10 minutes**, or until the solution clears.

a. The supernatant may be brownish in color due to residual blood components.

b. For sample volume larger than 200 µL, leave the sample plate on the magnet for additional 5 minutes for better separation.

10 Aspirate and discard the supernatant while the plate is situated on the magnet. Avoid disrupting the beads.

11 Repeat steps 8 through 10 for a second wash with the **Wash** WBB.

a. During the second wash, the beads will not clump as much as in the first wash. Mixing well is critical at this step as **Wash** WBB helps to rinse away digested protein. Incomplete resuspension may cause beads to clump together during the final elution step, which can make transfer of the eluant difficult.

12 Take the plate off the magnet. Add **1600 µL** of **Wash** WBC as prepared in Sample Preparation step 1, and pipette tip mix 10 times (with a **1 mL** pipette set to **0.8 mL**) or until the magnetic beads are completely resuspended from the bottom of the well.

NOTE The volume of **Wash WBC** does not vary based on the input amount and is dependent on the plate type used. Larger volume labware types may require increased wash volume. Resuspending the beads in **800 µL** first before adding an additional **800 µL** may make tip mixing easier.

13 Place the plate back on the magnet for **8 minutes**, or until the solution clears.

14 Aspirate and discard the supernatant while the plate is situated on the magnet.

15 Repeat steps 12-14 for a total of two WBC washes.

NOTE Remove as much of the final wash buffer as possible. Drying samples is not recommended for this protocol, as over-drying the DNA onto the beads make it difficult to fully elute the samples. Use a smaller pipette to remove any remaining visible liquid, if necessary. If the beads appear very wet, allow to dry at room temperature for up to five minutes, being careful that the beads do not over-dry and crack. Elute the samples before the beads appear cracked.

16 Add **elution buffer.**

a. **For BLOOD:** Add **200 µL** of **elution buffer** (1 x sample volume) to each sample to elute.

To determine volume, match the sample volume.
Example: 200 µL sample volume = 200 µL elution buffer.

Example Sample Volume	Volume of Elution Buffer
200 µL	200 µL
400 µL	400 µL

b. **For SERUM:** Add **40 µL** of **elution buffer** to each sample to elute.

NOTE Use smaller elution volumes if higher DNA concentrations are desired.

17 Remove the plate from the magnet and resuspend the beads by gently pipette tip mixing. Incubate the plate for **2 minutes** at **room temperature**, and then pipette **tip mix again** to complete the elution.

18 Place the plate back on the magnet for **2 minutes**, or until the supernatant clears. Transfer the supernatant to a clean plate or clean tubes.

NOTE If beads are being aspirated during the transfer, dispense the sample back into the well and let the plate sit for an additional **2 minutes** to better compact the beads. During the transfer, place the pipette tip in the center of the well or opposite side of the magnet bar and aspirate slowly.

Troubleshooting Guide

This troubleshooting guide may be helpful to maximize nucleic acid yield, integrity, and purity from blood/serum/cells, or to address issues that may arise when extracting nucleic acids using GenFind V3. The scientists at Beckman Coulter Technical Support is available to answer any questions you may have regarding the information contained in this troubleshooting guide and the protocols provided (please refer to contact us on page ii for contact information).

This section includes the following tables:

- Table 1, Troubleshooting Low Yield
- Table 2, Troubleshooting Poor Quality of Extracted Nucleic Acids (Low ratios of 260/230 and 260/280 UV absorbance)

Table 1 Troubleshooting Low Yield

Possible Causes	Possible Solutions and Comments
Poor Starting Sample Quality	• Improper handling/storage of the whole blood or serum, such as improper storage temperature or repeated freezing and thawing, might lead to poor quality of the starting material and/or clumping of the bead during Bind and incomplete resuspension of the beads during wash step due to insufficient digestion and results in sample loss and low yield (see **Bead/Sample Loss** and **Bead Clumping**).
Bead/Sample Loss	• Disruption of the bead pellet during supernatant removal steps may cause decreased yields. The pipette tip should not contact the bead pellet during aspirations. If beads are seen in the pipette tip during aspiration, the material in the pipette tip should be dispensed back into the tube or well and the samples should be placed back on magnet until the beads are fully settled towards the magnet before aspirating again. • Failure to allow beads to fully separate on the magnet may lead to decreased yields. Whole blood samples of poor staring quality (old, hemolyzed, lipemic, icteric or contaminated blood) should be given longer than 10 min during the Bind separation time to ensure that the beads are completely settled to the magnet before removing the supernatant. Increasing the lysis temperature to 37 - 55°C or the incubation time may help prevent clumping with particularly challenging samples. • Insufficiently digested proteins can trap the beads and prevent efficient nucleic acid binding, cause bead clumping and disrupt settling, or lead to bead and sample loss.

Table 1 Troubleshooting Low Yield *(Continued)*

Possible Causes	Possible Solutions and Comments
Bead Clumping	• Insufficient washing and inadequate removal of impurities can cause bead clumping. Ensure that the Wash steps are performed correctly. Consider adding additional Wash steps. • Excess input material and/or insufficient lysis can cause bead clumping. Consider performing an input titration to optimize starting sample quantity and/or altering lysis times or temperature.
Inaccurate Ethanol Percentage Used	• Ethanol is hygroscopic and may become more dilute over time; fresh pure ethanol should be used for Wash WBC assembly. Lower ethanol concentrations may increase solubilization of nucleic acids during Wash steps leading to loss of yield.
Over-dried beads	• Bead pellets that are over-dried prior to the Elution step may appear cracked and nucleic acids may bind too tightly to the beads to elute in water, causing low yield. Carefully monitor drying to ensure cracking does not occur and shorten dry time if needed.
Incomplete Elution	• Ensure that the recommended time and temperature are used during the Elution step to completely elute the nucleic acids off of the beads. • Consider performing a heated elution at 37-55^0 C.
Using non-recommended tube or plate types	• Different types of plastics can have variable rate of heat transfer resulting in unexpected in-well incubation temperatures. Adjust settings on heat sources to maintain specified in-well/tube temperatures. • Different types of plastics can cause variation in the magnetic field applied to the paramagnetic beads. Adjust settling times during bead separation steps to compensate for magnetic field strength.
Using non-recommended magnets	• Development of GenFind V3 was performed with the specific magnets listed in the recommended hardware section of this protocol. If using a non-recommended magnet, settling times may vary. Adjust settling times during bead separation steps; supernatant should be clear and pellet should be visible on the side wall of tube or well.

Table 2 Troubleshooting Poor Quality of Extracted Nucleic Acids (Low ratios of 260/230 and 260/280 UV absorbance)

Possible Causes	Possible Solutions and Comments
Using non-recommended tube or plate type	• Development of GenFind V3 was performed with the specific labware listed in the recommended hardware section of this protocol. If using a non-recommended plate type, adjust the volume of Wash WBC to make sure the wash buffer can cover the full well for sufficient contaminant removal.
Bead Clumping	• Insufficient washing and inadequate removal of impurities can cause bead clumping. Ensure that beads are completely resuspended during Wash steps. Consider adding additional Wash steps. • Excess input material and/or insufficient lysis can cause bead clumping. Bead clumping can trap contaminants, which can then be carried through to elution. Consider performing an input titration to optimize starting sample quantity and/or altering lysis times or temperature.

Table 2 Troubleshooting Poor Quality of Extracted Nucleic Acids (Low ratios of 260/230 and 260/280 UV absorbance) *(Continued)*

Possible Causes	Possible Solutions and Comments
Insufficiently mixing during Wash steps	• During both washes with WBB and WBC, make sure the beads are mixed well and sufficiently re-suspended. During the WBC wash step, resuspending the beads in half of the required volume first before adding the other half of the buffer may make tip mixing easier.
Contaminants carry over in Elution step	• Remove as much of the remaining liquid in the final Wash separation step as feasible before the Elute step to ensure minimal carryover of contaminants to the elution buffer. May consider adding a dry time (do not over-dry the bead pellet) if desired.

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