



Precipio, Inc.

Extraction Protocol

Extraction of DNA from plasma using the QIAamp®
Circulating Nucleic Acid Kit

Table of Contents

Notes for this Assay	2
Reagents and Equipment Required but not Supplied	2
Workplace Preparation	3
Plasma DNA Extraction Protocol	3

Copyright © 2018 by Precipio, Inc.

All rights reserved. This Instruction manual or any portion thereof may not be reproduced in any form, or transmitted outside of the recipient's organization in any form by any means — electronic, mechanical, photocopy, recording, or otherwise — or used in any manner whatsoever without the express written permission of the Company

Notes for this Assay

- The following protocol is presently used at Precipio, Inc. for for purifying circulating DNA from plasma/serum using the using the QIAamp® Circulating Nucleic Acid Kit.
- For further reference, please see the manual below:



QA_CirculatingNucAc
id_1013_WW.pdf

- The resulting extracted DNA can then be used in any of the Precipio ICE COLD-PCR enrichment kits.

Reagents and Equipment Required but not Supplied

Equipment:

1. QIAvac 24 Plus vacuum manifold: Qiagen Catalog # 19413
2. QIAvac Connecting System: Qiagen Catalog # 19419 or equivalent
3. QIAvac VacValves: Qiagen Catalog # 19408 or equivalent
4. Vacuum Pump: Qiagen Catalog # 84010 [USA and Canada], 84000 [Japan], or 84020 [rest of world] or equivalent pump capable of producing a vacuum of –800 to–900 mbar
5. Calibrated Pipettes
6. Sterile pipet tips (pipet tips with aerosol barriers are recommended to help prevent cross-contamination)
7. Water bath or heating block capable of holding 15 and/or 50 mL centrifuge tubes at 60°C
8. NIST Traceable thermometer
9. NIST Traceable Digital Timer, Cole Parmer Catalog # 94460.55
10. Heating block or similar at 56°C (capable of holding 2 mL collection tubes)
11. Microcentrifuge for 1.7 mL tubes
12. Centrifuge, Sorvall Legend X1 or equivalent
13. Vortexer
14. Spectrolinker (UV Crosslinker)
15. Tissue culture hood with germicidal light or PCR workstation with short-wave UV light or equivalent
16. Real-Time PCR instrument

Reagents:

1. QIAamp Circulating Nucleic Acid Kit: Qiagen Catalog # 55114
2. KAPA hgDNA Quantification and QC Kit (for specific model of real-time PCR instrument)
3. 15 or 50 mL conical tubes: VWR Catalog # 21008-918 or 21008-951
4. 5 mL centrifuge tubes: Eppendorf Catalog # 0030119460
5. 1.7 mL microfuge tubes: VWR Catalog # 29443-259
6. Ethanol (96–100%): Sigma Aldrich Catalog # E7023
7. Isopropanol (100%): Sigma Aldrich Catalog # I9030
8. Crushed ice