



Please see our website (catalog.coriell.org) for further information.

1. How is DNA extracted?

Genomic DNA is purified from fresh blood, immortalized lymphocytes or fibroblasts using the Qiagen Autopure LS instrument according to the manufacturer's instructions (whole blood, small scale lymphocytes, fibroblasts) or manually by modified Miller's Salting out procedures (large scale lymphocytes). Briefly, cells from whole blood or cultured cells are lysed by addition of anionic detergent, and RNA and protein are degraded with the addition of RNase and Proteinase K. After mixing, Autopure Precipitation Solution (Autopure) or a salt solution (Miller Salt Out) is added, and the insoluble cell debris is removed by centrifugation. An equal volume of isopropanol (Autopure) or ethanol (Miller Salt Out) is added to the supernatant, and the resulting DNA precipitate is collected by centrifugation (Autopure) or spooling (Miller Salt Out). Following a brief rinse with 70% ethanol to remove residual salt, the DNA pellet is solubilized in TE buffer (10 mM Tris, pH 8.0/1 mM EDTA).

2. How is DNA quality control performed?

After extraction, the DNA proceeds through several processing and quality control steps. DNA is solubilized over several days. The process is monitored by absorbance at 260 nm or the Qubit dsDNA BR Assay (please see #9 for full details), and the sample is considered fully hydrated when the values of two absorbance readings taken at least 1 day apart differ by no more than 10%. The last reading is recorded in the database along with the 260/280 nm absorbance ratio.

- To assess DNA integrity, ~2.0 µg of hydrated DNA is subjected to digestion with the restriction enzymes EcoRI and Hind III. Fragmented DNA products from these reactions along with undigested DNA are fluorescently stained and evaluated by the Agilent TapeStation. The TapeStation software determines a DNA Integrity Number (DIN) as a measure of gDNA integrity. (Note: This is not performed for all repositories.)
- DNA sample identity is confirmed using a multiplex PCR assay for 6 autosomal microsatellite markers. Gender is determined in the same reaction using an additional primer pair designed to amplify a region of allelic difference between the X and Y-chromosome amelogenin genes. Details of this assay and its importance in the quality control process are discussed below. This assay provides several quality control parameters as follows:
 1. It qualifies the DNA as a substrate for PCR.
 2. It provides a DNA fingerprint that is specific for the sample and the subject from which it is derived.
 3. It determines the gender of the subject from which the DNA is derived.

The criteria for acceptance of the DNA sample based upon all quality control procedures are as follows:

- Samples have a 260/280 ratio greater than or equal to 1.65 and less than 2.1. The concentration must be at least 0.1 mg/ml.
- Confirmed identity of sample donor.
- DNA must be intact, be of high molecular weight, and have a DIN score ≥ 7.0 as determined by the Agilent TapeStation (if specified by repository).

3. What is DNA dissolved in?

DNA is dissolved in TE buffer (10 mM TRIS pH 8.0, 1 mM EDTA, pH 8.0).

4. How much DNA is shipped in the vial?

The amount of DNA shipped in the vial is determined by the repository, and the method of quantitation also differs by repository. Please see #9 for specific information. This quantity of DNA is included on the catalog page for each item as well as the pricing page for each collection.

5. What is the concentration and volume of the DNA sample?

Most samples shipped by Coriell are between 300 – 375 ng/µl, but this differs by repository. The concentration for each DNA sample is available on the Concentration Sheet that accompanies all shipments. Please note that concentration is determined by different methods for different repositories. Please see #9 for specific information.



The volume in each DNA sample is determined by the following formula: $\text{Volume} = \text{Quantity of DNA} / \text{concentration}$

6. How is the DNA concentration determined?

DNA concentration is determined using either spectrophotometric absorbance at A260 using the convention that 1 O.D. is equivalent to 50 µg/ml of double-stranded DNA or the Qubit dsDNA BR assay, a dye-based fluorescence method, depending on the repository. Please see #9 for specific information. Samples are read at least two times to verify reading. Instruments are tested quarterly and calibrated as necessary.

7. How is DNA shipped?

DNA in tubes is shipped at ambient temperature and is stable. Please freeze at -20°C upon arrival if the sample will not be used shortly. DNA plates are shipped on dry ice.

8. How should DNA be stored?

DNA samples can be stored indefinitely at -80°C. Repeated freeze/thaw cycles should be avoided to reduce sample degradation. We recommend that you aliquot into sample sizes that will be adequate for future use in the lab.

9. How should I retest the DNA concentration?

We recommend using a Nanodrop or conventional cuvette-based spectrophotometer with TE as the appropriate sample blank. DNA concentration can be calculated from the A260 value using the convention that 1 O.D. is equivalent to 50 µg/ml of double-stranded DNA.

Qubit® dsDNA BR Assay (Q32853P7589; Invitrogen, Inc.) can also be used to determine DNA concentration. This assay uses an ultra-sensitive fluorescent nucleic acid stain for quantifying double-stranded DNA (dsDNA) in solution with a standard spectrofluorometer and fluorescein excitation and emission wavelengths.

The majority of Coriell DNA samples have been quantitated by A260. NHGRI and NIGMS samples extracted after August 1, 2011 only have been quantitated by the Qubit assay (refer to the table below). Please see the Concentration sheet included with your DNA to find out the date of extraction.

If you require that concentration be determined by an additional method by Coriell (or require a Certificate of Analysis for another method of quantitation), please inquire through Coriell Customer Service (customerservice@coriell.org).

Repository	Method of DNA Quantitation
NINDS	A260
NIGMS	Qubit as of August 1, 2011
NHGRI	Qubit as of August 1, 2011
NIA	Qubit as of August 1, 2011
NEI-AREDS	A260
HD Community, Autism, IPBIR, COHORT, YERKES, USIDNET, CDC, LMS, HuRef	A260

10. How are the DNA vials labeled?

Most vials will have a barcode label with lot number and the date extracted.