

DNAgard[®] Blood

Handbook

Room temperature preservation of DNA in whole blood

For room temperature storage and shipment of DNA in whole blood samples.

Biomātrica[®]
THE BIOSTABILITY COMPANY

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DNAgard® Blood is designed for shipping and storage of DNA in mammalian whole blood. DNA in such samples is protected by the unique stabilization properties of the DNAgard Blood formulation. Whole blood samples can be shipped and stored at room temperature, removing the need for frozen shipment and storage or immediate processing. DNAgard Blood is easy to use – blood samples and the DNAgard Blood solution are mixed together and stored at room temperature for at least 8 months. Samples stored in DNAgard Blood can be processed for DNA recovery via standard lab procedures (see the sample recovery section). Extracted and purified DNA can be used directly in any downstream application.

Kit Components

- DNAgard Blood (50 ml or 100 ml bottle)*
- Protocol

Storage

DNAgard Blood must be stored at room temperature. Use within 12 months of purchase date for optimal product performance.

DNAgard Blood stabilizes genomic DNA in mammalian whole blood for at least 8 months at room temperature.

Product Use Limitations

The DNAgard Blood test kit is for research use only. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease.

Safety Information

DNAgard Blood contains chaotropic salt and other chemicals that are harmful if swallowed. It is also irritating to eyes and skin (R22-R36/38, S13/26/36/46) if in contact. Keep away from food and drink. Wear suitable protective clothing and gloves when handling the product. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. If swallowed, seek medical advice immediately.

CAUTION: DNAgard Blood contains chemicals that form highly reactive compounds when combined with bleach. **DO NOT add bleach or acidic solutions directly to the sample-preparation waste.**

**Additional format under consideration (or in testing)*

SAMPLE COLLECTION

1. Add DNAgard Blood formulation to the whole blood specimen at a final ratio of 1:4 (formulation to blood). *For example, add 1ml DNAgard Blood to 4ml of whole blood.* To facilitate efficient mixing of stabilizing reagent and sample it is important that the final volume of the mixture not exceed 2/3 the fill capacity of the storage vessel.

DNAgard Blood reagent can be added directly to an evacuated blood collection tube (e.g., Vacutainer[®] or Vacuette[®]), or pre-aliquoted into microfuge tubes or larger tubes (ex. Falcon tubes). The order of addition to the tubes does not matter.

2. Mix well by pulse vortexing or inversion (at least 8 inversions).

SAMPLE RECOVERY

Genomic DNA can be extracted from blood samples stored in DNAgard Blood by multiple techniques including: column-based methods, magnetic bead technologies and salting out protocols. We have confirmed compatibility with the following commercially available kits:

- QIAamp Blood Mini Kit (QIAGEN)
- FlexiGene DNA Kit (QIAGEN)
- ChargeSwitch gDNA Blood Kit (Life Technologies)

For highest yields we recommend using the QIAGEN FlexiGene DNA Kit.

In all cases, the **blood-stabilizer mixture** should be treated as a whole blood **sample** when calculating reagent volumes in the isolation protocol.

NB: Heat will induce protein precipitation in blood samples and result in possible protein co-purification with DNA when performing salting-out protocols, hence in order to obtain the cleanest DNA we recommend using column-based purification methods for samples exposed to high temperatures (≥ 37 °C). See Annex A for more information.

Frequently Asked Questions

Situation	Comment	Suggestion
<p>The blood looks solid or coagulated after storage or shipment.</p>	<p>The sample may have been exposed to high temperature (>37°C) during transportation or storage.</p>	<p>If performing DNA isolation directly, we recommend using column-based isolation methods for cleanest DNA.</p> <p>Refer to the section above for tips when using column or salting-out protocols</p> <p>Before making aliquots from the blood sample, vortex until the sample is homogenous.</p>
<p>Low yield of recovered DNA</p>	<p>Possible reasons include:</p> <ul style="list-style-type: none"> -Low concentration of leukocytes in the blood sample. -DNAgard Blood: whole blood mixture at sub-optimal ratio. -Choice of DNA isolation technique. 	<ul style="list-style-type: none"> - Leukocyte concentrations can vary 10-fold between donors. Try isolation from a larger volume of blood. -Ensure that the DNAgard Blood:whole blood ratio is 1:4 (v/v) and that the samples are mixed thoroughly by vortexing or inversion (at least 8x) prior to shipment or storage. -We have confirmed compatibility of DNAgard Blood-stored samples with the following DNA isolation kits: <ul style="list-style-type: none"> • QIAamp Blood Mini Kit and FlexiGene DNA Kit (QIAGEN) • ChargeSwitch gDNA Blood Kit (Invitrogen) • UltraClean DNA Blood Isolation Kit (MoBio) <p>For highest DNA yield for samples stored at room temperature, we</p>

Situation	Comment	Suggestion
		<p>recommend the FlexiGene DNA Kit. If samples have been exposed to temperatures $\geq 37^{\circ}\text{C}$ we recommend using column-based isolation methods to obtain the cleanest DNA.</p> <p>See Annex A for “Tips for improved DNA yield in samples exposed to high heat”.</p>
<p>DNA isolated by salting-out protocol co-purifies with particulate matter.</p>	<p>The blood specimen was likely exposed to high temperatures ($\geq 37^{\circ}\text{C}$)</p>	<p>We recommend using column-based isolation methods to obtain the cleanest DNA for blood samples exposed to high temperatures.</p> <p>See Annex A for “Tips for improved DNA yield in samples exposed to high heat”.</p>
<p>Can I add DNAgard Blood formulation directly to blood collected in an evacuated blood collection tube?</p>	<p>Yes</p>	<p>Be sure to add DNAgard Blood at a volume ratio of 1:4 (formulation: blood) and mix well.</p>
<p>Do I have to store my blood sample at room temperature in DNAgard Blood?</p>	<p>Yes. Your blood sample is stabilized in DNAgard Blood for at least 8 months.</p>	<p>However, it is ok to store stabilized samples in the refrigerator (4°C) for at least 28 days or freezer (-20°C) for up to 4 months if desired.</p>

Annex A: Tips for improved DNA yield in samples exposed to high heat

We recommend the use of column-based DNA purification methods for blood samples exposed to high temperatures ($\geq 37^\circ\text{C}$).

Column-based DNA isolation: Exposure to high heat can result in blood samples with increased viscosity. To facilitate DNA purification, samples can be manually mixed with a sterile pipet tip after the addition of the kit's first lysis buffer. Sample heating for the digestion reaction and all subsequent steps are performed according to the kit protocol.

Salting-out methods of DNA isolation: Exposure to high heat can result in blood samples with increased viscosity. To facilitate DNA purification, samples can be manually mixed with a sterile pipet tip after the addition of the kit's first lysis buffer. Because of protein precipitation in heated blood samples, pellet sizes in salting-out protocols will likely be larger than in samples not exposed to high temperatures. Higher DNA yields can be achieved by increasing reagent volumes by the volume of the pellet as shown in the following example:

Example demonstrating reagent volumes for QIAGEN's FlexiGene DNA Kit for isolation of 300 μl whole blood-DNAGard Blood samples exposed to high heat compared with samples stored under standard (room temperature or below) conditions:

	Storage/ shipment at room temperature or below ($\leq 25^\circ\text{C}$)	Storage/ shipment at high temperature ($\geq 37^\circ\text{C}$)
Buffer FG1	750 μl	750 μl
Centrifugation/ discard supernatant \rightarrow pellet size	tiny	50 μl (for example)
Buffer FG2/ Protease	150 μl	200 μl (150 + 50 μl)
Heat at 65°C , 5 min.	yes	yes
Isopropanol (100%)	150 μl	200 μl (150 + 50 μl)
Remainder of precipitation and rehydration protocol	same	same

Technical Assistance

Biomatrica, Inc. takes pride in providing efficient quality technical support. Biomatrica's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of Biomatrica's biostability and storage products. Please contact Biomatrica directly with any questions regarding DNAgard technology, product use, or general matters.

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