Sera-Xtracta™ HMW DNA Kit

FAQs

Applications and automation

1. **What is Sera-Xtracta™ HMW DNA Kit?**
   The Sera-Xtracta™ HMW DNA Kit is designed for reproducible extraction of high-molecular-weight (HMW) DNA from a wide range of sample types including whole blood (treated with citrate, heparin or EDTA), buffy coat, saliva, cultured mammalian cells and solid tissue samples. The extraction protocols are designed to efficiently remove contaminants and PCR inhibitors while minimizing shearing, resulting in high quality high molecular weight DNA with minimal RNA carryover.

2. **With which applications is the Sera-Xtracta™ HMW Kit compatible?** The kit is compatible with most molecular biology techniques, including cloning, restriction enzyme digestion, PCR amplification, genotyping applications and next-generation sequencing.

3. **Are there any limitations on the sample types and volumes that can be extracted with Sera-Xtracta™ HMW DNA Kit, and what are the yields?** The kit has been tested for high purity extraction of genomic DNA from whole blood, buffy coat, saliva, cultured mammalian cells and solid tissue at a range of sample volumes. Please refer to the datafile for recommended input amounts for each sample type and the IFU for details of protocols at different input volumes. Scaling up sample volumes will affect the number of reactions per kit.

4. **Does the Sera-Xtracta™ HMW DNA Kit use chaotropic salt chemistry for DNA extraction?** Yes. The developed method uses chaotropic agents to extract DNA from a range of sample types, denature protein components and promote the selective binding of DNA to the silica-coated magnetic beads.

5. **Does Sera-Xtracta™ HMW DNA Kit contain superparamagnetic particles?**
   Yes, silica-coated magnetic beads are included.

6. **What is the protocol duration?**
   The protocol duration is 90 minutes.

7. **Is the Sera-Xtracta™ HMW DNA Kit automation-friendly?**
   Yes; specific scripts are available for KingFisher™ Duo. Please contact Scientific Support (cytiva.com/support/contact-us). When automating, pay attention to the settling time and employ intermittent mixing if necessary.

8. **Is the Sera-Xtracta™ HMW DNA Kit compatible with different blood collection tubes?** Yes, the kit is compatible with blood collected in EDTA, heparin and citrate tubes. To demonstrate the removal of inhibitors such as heme, anticoagulants, enzymes and divalent cations, whole blood from a single donor was collected in EDTA, heparin and citrate blood collection tubes. Data demonstrated a highly linear correlation of sample input volume with Ct values, indicating the absence of PCR inhibitors in DNA isolated from whole blood collected in all three blood collection tubes.

9. **Why is RNase treatment stated as optional in the protocol?**
   The Sera-Xtracta™ HMW DNA Kit has been designed to minimize co-purification of RNA. This simplifies the protocol, but for applications particularly sensitive to RNA, an option for RNase treatment is included.

Storage and handling

1. **How should Sera-Xtracta™ HMW DNA Kit be stored?**
   Store Sera-Xtracta™ HMW DNA Kit at room temperature (15°C–30°C). Proteinase K in liquid format can be stored at 2°C–8°C if desired.

2. **Why is Proteinase K included in the kit?** Proteinase K is the enzyme of choice for SDS-containing lysis buffers and is active even when enzyme inhibitors such as EDTA and detergents are present in samples.

3. **Is a pulse spin in a microcentrifuge necessary before magnetic settling?** A pulse spin in a microcentrifuge is strongly recommended before magnetic bead settling to ensure all the liquid sample in the tube is collected together in a single bulk volume at the bottom of the tube. Beads present in isolated droplets will not settle on the magnet and liquid will not be aspirated properly. If air-drying the DNA-bound beads to remove residual ethanol after washing is considered an issue, it is also possible to replace this step with 1 or 2 rinses with DNase-free water (700 µL). See IFU for details.

4. **Can the purified DNA be stored at room temperature?**
   Purified genomic DNA may be stored at 2°C–8°C for up to a week if being used directly for analysis and/or downstream molecular biology applications. In order to maintain a high-quality product for repeated use, aliquot and store purified samples at -20°C or less.

5. **Can DNase-free water be used for eluting samples?** The elution buffer provided in the kit should be the preferred buffer for eluting samples, although DNase-free water can be used. DNA eluted in water is not recommended for long-term storage since DNA undergoes acid hydrolysis.

6. **If I want to use more sample volume than recommended, is the protocol scalable?** Technically yes. See further information on scale-up volumes (up to 2 mL) in the user guide. Note that increasing the sample volume would require using suitable containers and magnets because higher amounts of beads and buffers will also be required for an efficient extraction.

7. **Do I need additional buffers for extraction of HMW DNA from cultured cells and tissues (lung or kidney), or buffy coat?** No. The protocol is optimized for extracting from these sample types without the need to purchase additional reagents to combine with the kit.

8. **Do the tissue and cell protocols require an RNase A treatment step?** RNase treatment is optional in the protocols. The kit is designed to minimize RNA carryover during the purification. If it is critical to obtain RNA-free product, we recommend to treat the starting sample with RNase A prior to the addition of the lysis buffer. A final concentration of 1–2 mg/mL RNase is sufficient to degrade RNA (e.g. 4 µL RNase A at 100 mg/mL per 200 µL sample input).

Physical characteristics

1. **Where can I find the Certificate of Analysis (CofA) for my lot of Sera-Xtracta™ HMW DNA?** Enter your specific lot number to retrieve the CofA at cytiva.com/certificates.

2. **How many reactions should be possible with one Sera-Xtracta™ HMW DNA Kit?** 96 purifications (completed in a plate or microfuge tube) when processing sample volumes up to 200 µL.

3. **What is included in the Sera-Xtracta™ HMW DNA Kit?**

Component	Quantity
Proteinase K	3 mL
Lysis buffer	22 mL
Silica-coated magnetic beads	1.5 mL
Binding buffer	26 mL
Wash buffer 1 AQ	120 mL
Wash buffer 2 AQ	30 mL
gDNA elution buffer	14 mL

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