Presto™ Sperm DNA Extraction Kit

For research use only

Catalogue Numbers

GSP100
GSP300

Quantity

100 rxns
300 rxns

CERTIFICATE NO. QAIC/TW/50077
ISO 9001:2008 QMS

Introduction

The Presto™ Sperm DNA Extraction Kit is optimized for DNA purification from animal semen samples. Proteinase K and a unique sperm lysis buffer are used to lyse sperm cells and degrade protein, allowing DNA to efficiently bind to the glass fiber matrix of the spin column. Contaminants are removed using a wash buffer and the purified DNA is eluted by a low salt elution buffer, TE or water. Following cell lysis, the procedure can be completed in as little as 10 minutes without phenol/chloroform extraction or alcohol precipitation. The purified DNA is suitable for use in PCR or a variety of other enzymatic reactions.

Quality Control

The quality of the Presto™ Sperm DNA Extraction Kit is tested on a lot-to-lot basis according to Geneaid's ISO-certified quality management system. Total DNA is isolated from 100 µl of animal semen samples, quantified with a spectrophotometer and analyzed by electrophoresis.

Specifications

- Sample: up to 100 µl of animal semen
- Binding Capacity: up to 50 µg of total DNA
- Elution Volume: 30-200 µl
- Operation Time: in as little as 10 minutes following cell lysis incubation
- Storage: dry at room termperature (20-25ºC)

Caution

GSB Buffer and W1 Buffer contain chaotropic salt. During the procedure, always wear a lab coat, disposable gloves and protective goggles.

Components

Product Name	GSP004	GSP100	GSP300
Sperm Lysis Buffer	900 µl	12 ml	35 ml
GSB Buffer	4 ml	40 ml	75 ml
W1 Buffer	2 ml	45 ml	130 ml
Wash Buffer[1] (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml (200 ml)
Proteinase K[2] (Add ddH_2O)	1 mg (0.1 ml)	11 mg (1.10 ml)	11 mg (1.10 ml)
Elution Buffer	1 ml	30 ml	75 ml
GS Column	4 pcs	100 pcs	300 pcs
2 ml Collection Tube	8 pcs	200 pcs	600 pcs

[1]Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

[2]Add ddH_2O to Proteinase K (see the bottle label for volume) then vortex to ensure it's completely dissolved. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin the mixture down. For extended periods, the ddH_2O and Proteinase K mixture should be stored at 4ºC. Use only fresh ddH_2O as ambient CO_2 can quickly cause acidification.

Sperm DNA Purification Procedure

Please read the entire instruction manual prior to starting the following procedure.

Additional Requirements

1.5 ml microcentrifuge tubes, 1M Dithiothreitol (DTT) solution.

1. Buffer Preparation

Transfer **900 µl of Sperm Lysis Buffer** into a 1.5 ml microcentrifuge tube. Add **20 µl of Proteinase K and 80 µl of 1M DTT solution*** immediately before use. Vortex to mix well.

*Dissolve 1.5 g of Dithiothreitol in 8 ml of deionized ddH_2O. Adjust volume to 10 ml. Dispense into 1 ml aliquots, and store at -20ºC. Thaw DTT solution to room temperature before use.

2. Cell Lysis

Add **100 µl of semen and 100 µl of fresh prepared Sperm Lysis Buffer (containing DTT and proteinase K)** into a new 1.5 ml microcentrifuge tube then vortex to mix well. Incubate at 60ºC for 1 hour to lyse the sperm cells.

NOTE: Inverting the sample occasionally during incubation will facilitate Proteinase K digestion and cell lysis. Using an auto shaking system during incubation is recommended.

During incubation, transfer the required volume of **Elution Buffer (200 µl/sample)** to a 1.5 ml microcentrifuge tube and heat to 60ºC (for Step 5 DNA Elution). Add **200 µl of GSB Buffer** then shake vigorously for 10 seconds.

NOTE: It is essential that the sample and GSB Buffer are mixed thoroughly to yield a homogeneous solution.

Optional RNA Removal Step

For RNA-free gDNA, following GSB Buffer addition, add 5 µl of RNase A (50 mg/ml) and mix by shaking vigorously. Incubate at room temperature for 5 minutes to ensure efficient RNA degradation.

3. DNA Binding

Add **200 µl of of absolute ethanol** to the sample lysate and mix IMMEDIATELY by shaking the mixture vigorously for 10 seconds. If precipitate appears, break it up as much as possible with a pipette.

NOTE: It is important that the lysate and ethanol are mixed thoroughly to yield a homogeneous solution.

Place a **GS Column in a 2 ml Collection Tube**. Transfer all of the mixture (including any insoluble precipitate) to the GS Column. Centrifuge at 14-16,000 x g for 1 minute. Following centrifugation, if the mixture did not flow through the GS Column membrane, increase the centrifuge time until it passes completely. Discard the 2 ml Collection Tube containing the flow-through then transfer the GS Column to a new 2 ml Collection Tube.

4. Wash

Add **400 µl of W1 Buffer** then centrifuge at 14-16,000 x g for 30 seconds. Discard the flow-through then place the GS Column back in the 2 ml Collection Tube. Add **600 µl of Wash Buffer (make sure ethanol was added)**. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. Place the GS Column back in the 2 ml Collection Tube and centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix.

5. Elution

Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl.

Transfer the dry GS Column to a clean 1.5 ml microcentrifuge tube. Add **100 µl of pre-heated Elution Buffer**[1], TE Buffer[2] or water[3] into the CENTER of the column matrix. Let stand for at least 3 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed. Centrifuge at 14-16,000 x g for 30 seconds to elute the purified DNA.

[1]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) is added into the center of the GS Column matrix and is completely absorbed.

[2]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the GS Column matrix and is completely absorbed.

[3]If using water for elution, ensure the water pH is between 7.0 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the center of the GS Column matrix and is completely absorbed. DNA Eluted in water should be stored at -20ºC to avoid degradation.

Troubleshooting

1. Incomplete buffer preparation: Add absolute ethanol to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation. Add ddH_2O pH7.0-8.5 to Proteinase K then vortex to ensure it is completely dissolved. Check the box on the bottle. For extended periods, the Proteinase K mixture should be stored at 4ºC. Add 20 µl of Proteinase K and 80 µl of 1M DTT solution into Sperm Lysis Buffer immediately before use.

2. Residual ethanol contamination: Following the wash step, dry the GS Column with additional centrifugation at 14-16,000 x g for 5 minutes to ensure the GS Column mebrane is completely dry.

3. Residual RNA Contamination: Perform the optional RNA removal step.

4. Degraded DNA: Use fresh semen samples and store the purified DNA at -20ºC.