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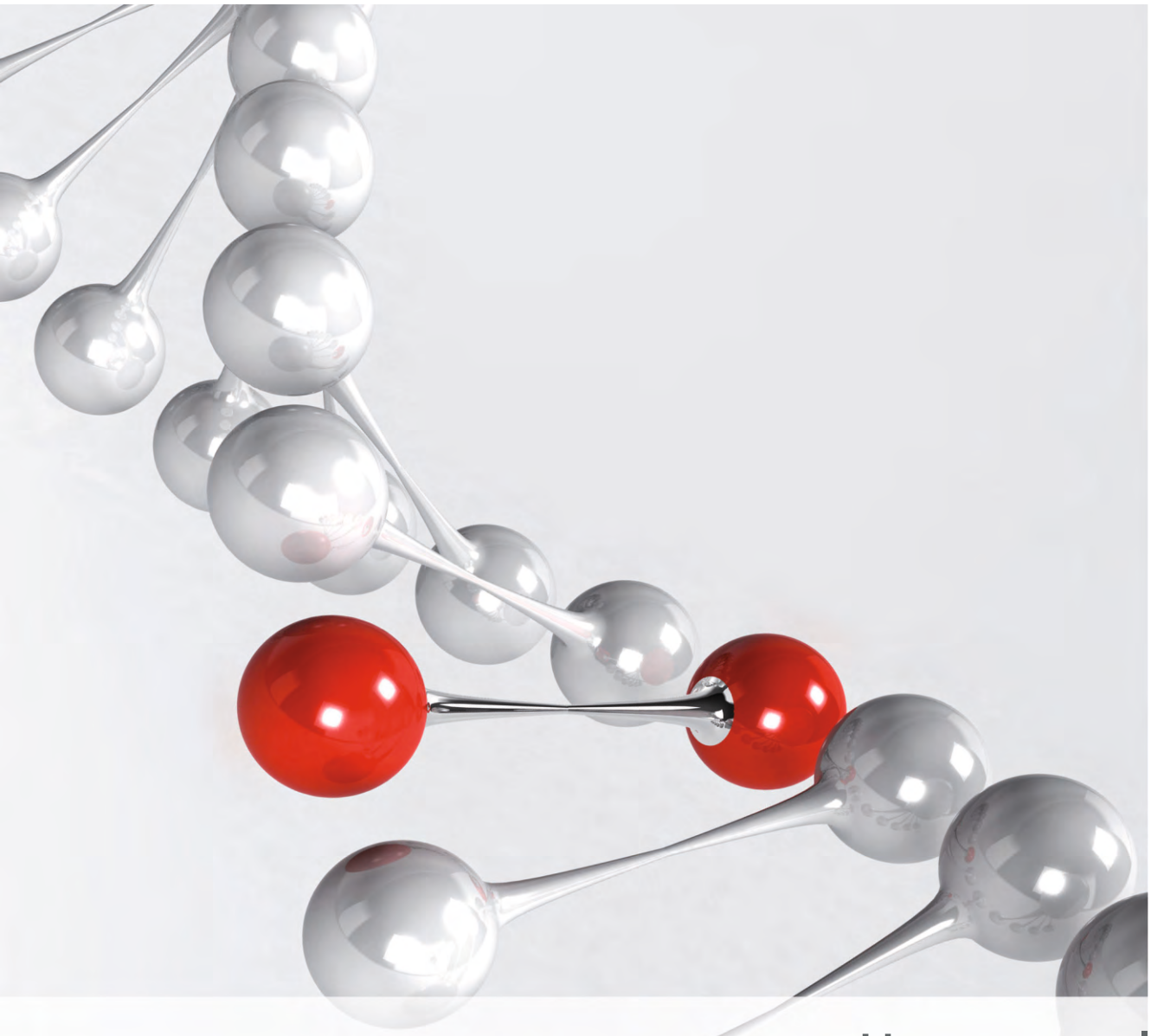


User manual
InviGene® Bisulfite Conversion Kit

for bisulfite conversion of DNA (human genomic DNA from different sources)

REF 3030100100 STRATEC Molecular GmbH, D-13125 Berlin

Instruction for InviGene® Bisulfite Conversion Kit

The **InviGene® Bisulfite Conversion Kit** is the ideal tool for Bisulfite conversion of DNA (human genomic DNA from different sources).

Due to the high purity, the converted DNA is ready for use in methylation analysis or can be stored at -20°C/ -80°C for subsequent use.

Contents

Kit contents of the InviGene® Bisulfite Conversion Kit

	10 Conversion	50 Conversion
Catalogue No.	3030100900	3030100100
Solution BS	2 ml	4 x 2 ml
Binding Buffer BS	60 ml	60 ml
Wash Buffer M	31 ml	31 ml
Solution D	10 ml	10 ml
Elution Buffer	2 ml	2 ml
RTA Spin Filter Set	10	50
RTA Receiver Tubes	30	3 x 50
2.0 ml Safe-Lock-Tubes	10	50
1.5 ml Receiver Tubes	2 x 5	50
Initial steps	Add 95 ml 96-100% ethanol to the **Wash Buffer M** Add 30 ml 96-100% ethanol to the **Solution D**	Add 95 ml 96-100% ethanol to the **Wash Buffer M** Add 30 ml 96-100% ethanol to the **Solution D**

Symbols

 Manufacturer

 Lot number

Attention: *Do not combine components of different kits, unless the lot numbers are identical!*

 Catalogue number

 Expiry date

 Consult operating instructions

 Temperature limitation

 Do not reuse

 Humidity limitation

Storage

All buffers and kit contents of the **InviGene Bisulfite Conversion Kit** should be stored dry, at room temperature and are stable for at least 6 months

Room temperature (RT) is defined as range from 15-30°C.

Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions solve these precipitates by warming carefully (up to 30°C).

Quality control and product warranty

STRATEC Molecular warrants the correct function of the **InviGene® Bisulfite Conversion Kit** for applications as described in this manual. Purchaser must determine the suitability of the product for its particular use. Should any product fail to perform the applications as described in the manual, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a problem in the lot, STRATEC Molecular will replace the product free of charge.

STRATEC Molecular reserves the right to change, alter, or modify any product to enhance its performance and design at any time.

In accordance with STRATEC Molecular's ISO 9001 and ISO EN 13485 certified Quality Management System the performance of all components of the **InviGene® Bisulfite Conversion Kit** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

If you have any questions or problems regarding any aspects of **InviGene® Bisulfite Conversion Kit** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

For technical support or further information please contact:

from Germany: +49-(0)30-9489-2901/ 2910
from abroad: +49-(0)30-9489-2903/ 2907
or contact your local distributor.

Intended use

The **InviGene® Bisulfite Conversion Kit** is designed for DNA denaturation and bisulfite conversion processes into one-step using the RTA Spin Filter system.

DNA methylation is an important regulator of gene transcription and a large body of evidence has demonstrated that genes with high levels of 5-methylcytosine in their promoter region are transcriptionally silent, and that DNA methylation gradually accumulates upon long-term gene silencing. DNA methylation is essential during embryonic development, and in somatic cells, patterns of DNA methylation are generally transmitted to daughter cells with a high fidelity. Aberrant DNA methylation patterns – hypermethylation and hypomethylation compared to normal tissue – have been associated with a large number of human malignancies. Hypermethylation typically occurs at CpG islands in the promoter region and is associated with gene inactivation. A lower level of leukocyte DNA methylation is associated with many types of cancer. Global hypomethylation has also been implicated in the development and progression of cancer through different mechanisms. Typically, there is hypermethylation of tumor suppressor genes and hypomethylation of oncogenes.

In prokaryotes DNA methylation provides a way to protect host DNA from digestion by restriction endonucleases that are designed to eliminate foreign DNA, or from destruction during repair events. In higher eukaryotes DNA methylation functions in the regulation/control of gene expression. It has been demonstrated that aberrant DNA methylation is a widespread event in cancer. A changed methylation pattern may be among the earliest changes to occur during oncogenesis. DNA methylation has also been shown to play a central role in gene imprinting, embryonic development, X-chromosome gene silencing, and cell cycle regulation. In eucaryotik CpG - DNA methylation consists of the addition of a methyl group to the fifth carbon position of the cytosine pyrimidine ring via a methyltransferase enzyme. The majority of DNA methylation in mammals occurs in CpG' dinucleotides, but other methylation patterns do exist. In fact, 70 - 80 percent of all CpG dinucleotides in mammalian genomes are found to be methylated, whereas the majority of the twenty to thirty percent that remain unmethylated are within promoters or in the first exons of genes.

Nowadays efficiently and accurate DNA methylation analysis is essential for the study of cancer, gene expression, genetic diseases, as well as many other important aspects of biology. To date, a number of methods have been developed to detect/quantify DNA methylation including: high-performance capillary electrophoresis and methylation-sensitive arbitrarily primed PCR. However, the most common technique used today remains the bisulfite conversion method. This technique involves treating methylated DNA with bisulfite, which converts unmethylated cytosines into uracil. Methylated cytosines remain unchanged during the treatment. Once converted, the methylation profile of the DNA can be determined by PCR amplification followed by DNA sequencing.

THE PRODUCT IS INTENDED FOR USE BY PROFESSIONALS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

Product use limitation

InviGene® Bisulfite Conversion Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

The product is only for Bisulfite conversion of DNA in low salt buffers or water, any other contaminant may interfere with the process.

Differing the starting material or flow trace may lead to inoperability. Therefore neither a warranty nor a guarantee in this case will be given, implied or expressed.

The user is responsible to validate the performance of the STRATEC Molecular product for any particular use. STRATEC Molecular does not provide validations of performance characteristics of the product with respect to specific applications.

STRATEC Molecular products may be used e.g. in clinical diagnostic laboratory systems under following conditions:

- If used in the US, based on the condition that the complete diagnostic system of the laboratory has been validated pursuant to CLIA' 88 regulations.
- For other countries based on the condition that the laboratory has been validated pursuant to equivalents according to the respective legal basis.

All products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001 and/or ISO EN 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.

The chemicals and the plastic parts are for laboratory use only. They must be stored in the laboratory and must not be used for other purposes than intended.

The included chemicals are only useable once and are not suitable for consumption.

Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!

Avoid skin contact! Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at **www.stratec.com** for each STRATEC Molecular product and its components. If buffer bottles are damaged or leaking, **WEAR GLOVES, AND PROTECTIVE GOGGLES** when discarding the bottles in order to avoid any injuries.

European Community risk and safety phrases for the components of the **InviGene® Bisulfite Conversion Kit** to which they apply are listed below as follows:

Solution BS

Danger

H314,317,412,P280,305+351+338

Solution D

Danger

H314, P280,305-351-338

H314: Causes severe skin burns and eye damage.
H317: May cause an allergic skin reaction.
H412: Harmful to aquatic life with long lasting effects.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.present and easy to do. Continue rinsing.

Emergency medical information can be obtained 24 hours a day from infotrac:

outside of USA: 1 – 352 – 323 – 3500
inside of USA: 1 – 800 – 535 – 5053

Product characteristics of the InviGene® Bisulfite Conversion Kit

The **InviGene® Bisulfite Conversion Kit** provides a fast and efficient way for Bisulfite conversion of high quality DNA. The procedures are suitable for use with DNA Eluate. Samples can be fresh or frozen.

Starting Material	Yield	Time for preparation
For optimal results, the amount of input DNA should be 500 pg to 5 µg (20-50 µl eluted DNA), if using low amounts, please think of dilution effects and use a low elution volume	depending on starting amount DNA Recovery >50% Conversion Efficiency >99%	about 90 min per bisulfite conversion reaction

Yield and quality of the converted DNA is suitable for PCR and methylation analysis by the respective methods (Real Time PCR, Sequencing, High Throughput Sequencing, Digital PCR and more).

For further information please contact: +49 (0) 30 9489 2901, -2910 in Germany and from foreign countries +49 (0) 30 9489 2903, -2907 or your local distributor.

Principle and procedure

The **InviGene® Bisulfite Conversion Kit** procedure comprises following steps:

- Denaturation and Bisulfite Conversion
- Binding of the DNA to the filter membrane and Desulfonation
- Washing the filter bound DNA and elimination of alcohol
- Elution of DNA

Important points before starting a protocol

Immediately upon receipt of the product, inspect the product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities you have to notify STRATEC Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to “Safety Information” (see page 6). Do not use damaged kit components, since their use may lead to poor kit performance.

- Always change pipette tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipette tips.
- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Discard gloves if they become contaminated.
- Do not combine components of different kits, unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- This kit should only be used by trained personnel.

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information please consult the appropriate material safety data sheets (MSDS). (See our webpage: www.stratec.com)

1. Microcentrifuge ≥ 18.000 x g (≥ 13.000 rpm)
2. Thermomixer (37°C - 95°C)
3. Ethanol (96-100%) *
4. Disposable gloves
5. Pipet with tips

*The **InviGene® Bisulfite Conversion Kit** is tested with **Ethanol** absolute, reag. ISO, reag. Ph. Eur., ≥99.8% (GC), liquid (clear, colorless) from **Sigma-Aldrich** (Order Nr.32205)

Important indications

Handling of RTA Spin Filter

Due to the sensitivity of DNA amplification technologies the following precautions are necessary when handling the RTA Spin Filter to avoid cross-contamination between sample preparation.

1. carefully apply the sample or solution to the RTA Spin Filter, pipet the sample onto the filter without wetting the rim of the column
2. always change pipet tips between liquid transfers, we recommend the use of aerosol barrier pipet tips
3. avoid touching the RTA Spin Filter membrane with the pipet tip

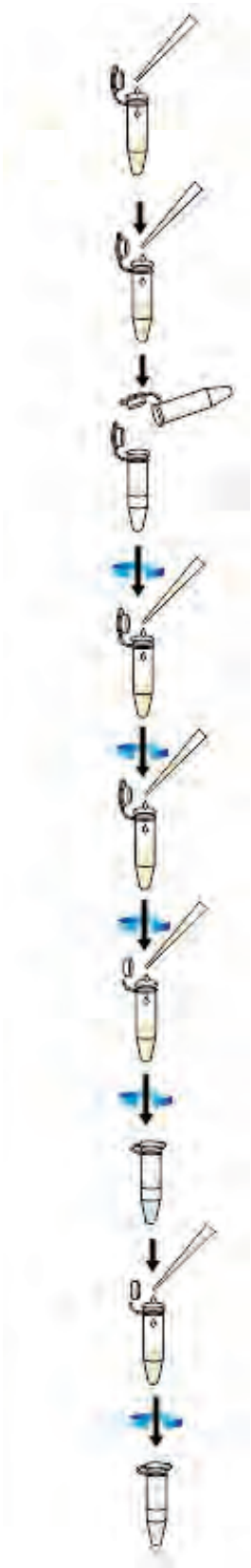
Storage of starting materials

Please read the instructions carefully and conduct the prepared procedure.

Sampling and storage

Storage of DNA
A working stock of DNA can be stored at 2 – 8˚C for several weeks. For long term storage DNA should be stored at -20˚C, but storing at – 20°C can cause shearing, particularly if the DNA is exposed to repeated freeze-thaw cycles.

Scheme of the InviGene® Bisulfite Conversion Kit



Please read the protocols carefully prior to the start of the preparation procedure!
Check that Wash Buffer M and Solution D are adjusted correctly

Preheat the **Elution Buffer** at 85°C 5 minutes prior to elution, do not switch off the Thermoblock after BS reaction

Transfer up to 5 µg (min. 20 µl; max.50 µl) sample DNA into the provided

2.0 ml Safe-Lock-Tube

Add 150 µl **Solution BS**
incubate for 60 minutes at 85°C in a thermomixer with 700 rpm

Add 1.000 µl **Binding Buffer BS**
vortex for 10 sec
incubate for 2-3 min at RT

Transfer **700 µl** sample into the provided **RTA Spin Filter**
centrifuge for 1 min at 11.000 x g (11.000 rpm)
Discard the flow-through and put the RTA Spin Filter back into a new RTA Receiver Tube.
Repeat this step with remaining sample

Add 700 µl **Wash Buffer M**
centrifuge for 1 min at 11.000 x g (11.000 rpm)
Discard the flow-through and put the RTA Spin Filter back into the used RTA Receiver Tube

Add 700 µl **Solution D**
to the **RTA Spin Filter**
incubate for 20 min at RT
centrifuge for 1 min at 11.000 x g (11.000 rpm)
Discard the flow-through and put the RTA Spin Filter back into a new RTA Receiver Tube

Add 700 µl **Wash Buffer M**
centrifuge for 1 min at 11.000 x g (11.000 rpm)
Discard the flow-through and put the RTA Spin Filter back into the used RTA Receiver Tube

Repeat this washing step once

Centrifuge for **5 min at 18.000 x g (13.000 rpm)** and discard the RTA Receiver Tube

Put the **RTA Spin Filte**r in a **1.5 ml Receiver Tube**

Pipet 30 µl (min 20 µl; max. 50 µl) from preheated
Elution Buffer directly on the middle of the RTA Spin Filter

Centrifuge for **1 min at 11.000 x g (11.000 rpm)**
Discard the RTA Spin Filter

Close the 1.5 ml Receiver Tube and store the eluated sample at 2-8 °C, for long term storage at -20°C.

Protocol: Bisulfite Conversion from DNA

Please read the protocols carefully prior to the start of the procedure!

Important Note: *You have to preheat the required amount of **Elution Buffer to 85°C** for 5 minutes before final elution step. Do not switch off the Thermoblock after BS reaction (step 1)*

Attention: *Before starting, control that Solution D and Wash Buffer M are diluted with Ethanol*, and quantify your purified DNA to find the optimal DNA concentration.*

1. **Denaturation and Bisulfite Conversion:**

   In a 2.0 ml Safe-Lock-Tube add 20-50 µl **purified DNA** (depending on concentration of DNA) and add 150 µl **Solution BS**. Place the Tubes into a thermomixer and incubate under continuously shaking (700 rpm) for 60 minutes at 85°C.

2. **Binding of the DNA**:

   Add 1.000 µl **Binding Buffer BS** to the sample and mix the sample completely by vortexing for 10 sec. Incubate the sample at room temperature for 2-3 minutes. Transfer 700 µl of the mixture into the provided RTA Spin Filter. Centrifuge for 1 minute at 11.000 x g (11.000 rpm). Discard the flow-through and put the RTA Spin Filter back into a new RTA Receiver Tube. Repeat this step with the remaining sample mixture.

3. **First Washing of the RTA Spin Filter:**

   Add 700 µl **Wash Buffer M** to the RTA Spin Filter and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the flow-through and put the RTA Spin Filter back into the used RTA Receiver Tube.

4. **Desulfonation:**

   Add 700 µl **Solution D** to the RTA Spin Filter and incubate for 20 min at Room temperature. Centrifuge for 1 min at 11.000 x g (11.000 rpm). Discard the flow-through and put the RTA Spin Filter back into a new RTA Receiver Tube.

5. **Second Washing of the RTA Spin Filter:**

   Add 700 µl **Wash Buffer M** to the RTA Spin Filter and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the flow-through and put the RTA Spin Filter back into the used RTA Receiver Tube.

   Repeat this washing step once.

6. **Ethanol removal:**

   Remove the residual ethanol by final centrifugation for 5 min at 18.000 x g (13.000 rpm).

7. **Elution of the DNA:**

   Discard the RTA Receiver Tube and place the RTA Spin Filter into a 1.5 ml Receiver Tube.

   Add 30 µl (lowest 20 µl; highest 50 µl) of the **preheated Elution Buffer** directly onto the RTA Spin Filter surface.

8. Centrifuge at 11.000 x g (11.000 rpm) for 1 minute. Discard the RTA Spin Filter. Close the 1.5 ml Receiver Tube and store the DNA sample for short term at 2-8°C, for long term storage at -20°C.

* Add 95 ml 96-100% ethanol to the **Wash Buffer M**

* Add 30 ml 96-100% ethanol to the **Solution D**

Troubleshooting

Problem	Probable cause	Comments and suggestions
Low concentration of extracted/converted DNA	Too much Elution buffer	Elute the converted DNA with lower volume of Elution Buffer (between 20-50 µl). Elution with a volume below 20 µl is not recommended due to drastic losses in yield
	Concentration of your starting Sample DNA is too low	ensure that the storage of starting material was correct avoid multiple freezing and thawing cycles of the material
Low conversion rate	Combination of reagents from different kits	Please make sure that only reagents belonging to one kit lot are used.
	DNA after Bisulfite conversion always is fragmented. Fragments should be long enough to allow Methylation detection via PCR and downstream reactions	Make sure that your starting material (DNA) is of sufficient quality

Appendix

General notes on handling DNA

Starting material
This kit is designed for Bisulfite conversion of purified DNA.

Nature of DNA
The length and delicate physical nature of DNA requires careful handling to avoid damage due to shearing and enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature, and UV irradiation. Careful isolation and handling of high molecular weight DNA is necessary to ensure compatibility with various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting, and long-template PCR.

Storage of Bisulfite converted DNA
A working stock of Bisulfite converted DNA can be stored at 2 – 8˚C for short time. For long term storage converted DNA should be stored at -20˚C.

Note that the solution in which the nucleic acid is eluted in will affect it's stability during storage. Pure water lacks buffering capacity and an acidic pH may lead to acid hydrolysis. Tris or Tris-EDTA buffer contains sufficient buffering capacity to prevent acid hydrolysis.

Drying, dissolving and pipetting DNA
Avoid over drying genomic DNA after ethanol precipitation. It is better to let it air dry than to use a vacuum, although vacuum drying can be used with caution.
Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings causes shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for pipetting genomic DNA.

Bisulfite Conversion of DNA Templates

The following illustrates what occurs to a DNA template during bisulfite conversion

Template:	**A:**	5′-**GACCGTTCCAGGTCCAGCAGTGCGCT**-3′
	B:	3′-**CTGGCAAGGTCCAGGTCGTCACGCGA**-5′
Bisulfite Converted:	**A:**	5′-**GATCGTTTTAGGTTTAGTAGTGCGTT**-3′
	B:	3′-**TTGGCAAGGTTTAGGTTGTTATGCGA**-5′

Note: Methylated "C" is underlined in the example

Ordering information

Product	Package Size	Catalogue No.
InviGene® Bisulfite Conversion Kit	10 conversion	3030100900
InviGene® Bisulfite Conversion Kit	50 conversion	3030100100

Possible suppliers for centrifuges:

Eppendorf AG
22331 Hamburg, Germany
Tel.: +49 (0) 40 53801 0
Fax: +49 (0) 40 53801 556
E-Mail: eppendorf@eppendorf.com
Internet: www.eppendorf.com

SIGMA Laborzentrifugen GmbH
37507 Osterode am Harz, Germany
Tel.: +49-5522-5007-0
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