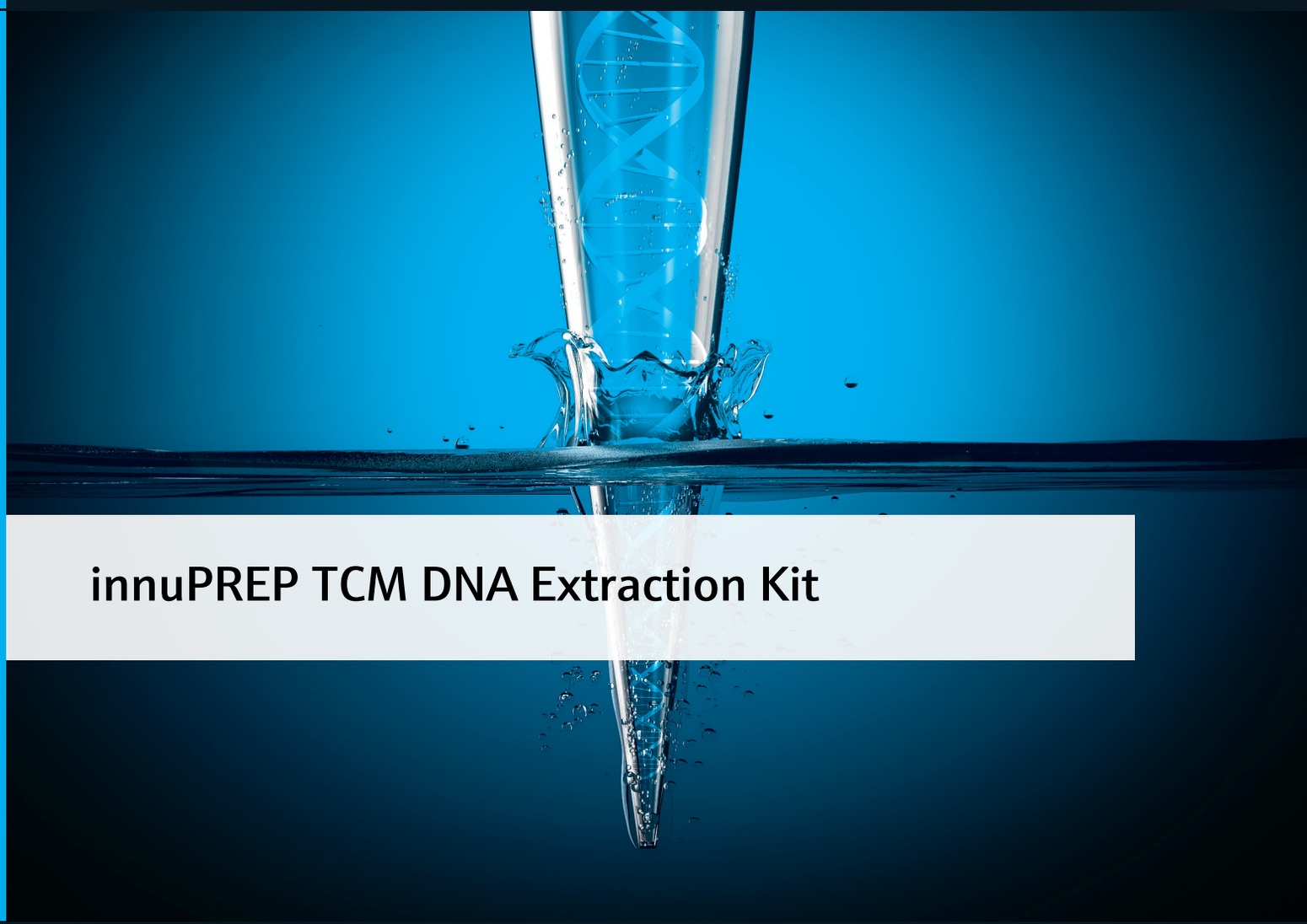
Instructions for Use
Life Science Kits & Assays



innuPREP TCM DNA Extraction Kit

Innuscreen GmbH

Order No.:
845-KS-3200050 50 reactions

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1 Introduction

1.1 Intended use

The **innuPREP TCM DNA Extraction Kit** has been designed as a very efficient tool for fast isolation of genomic DNA from different kinds of samples e.g. wood, plants, different kind of plants roots, beans etc.

The Kit includes a Lysis Tube for the efficient homogenization of the different kind of starting materials. The extraction procedure is based on a new patented chemistry and combines lysis of samples with subsequent binding of nucleic acids onto the surface of a Spin Filter membrane. After several washing steps the nucleic acids are eluted from the membrane by using Elution Buffer. Extraction chemistry and extraction protocol are optimized to get maximum of yield.

Furthermore, the kit includes a Re-binding protocol. This protocol could be used in the case of coloured eluates after first extraction procedure. The Re-binding protocol removes efficiently impurities from the coloured eluates.

CONSULT INSTRUCTION FOR USE
This package insert must be read carefully before use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.

1.2 Notes on the use of this manual and the kit

For easy reference and orientation, the manual and labels use the following warning and information symbols as well as the shown methodology:

Symbol	Information
REF	**REF** Catalogue number.
Σ N	**Content** Contains sufficient reagents for <N> reactions.
15°C 30°C	**Storage conditions** Store at room temperature or shown conditions respectively.
	Consult instructions for use This information must be observed to avoid improper use of the kit and the kit components.
	Expiry date
LOT	**Lot number** The number of the kit charge.
	Manufactured by Contact information of manufacturer.
	For single use only Do not use components for a second time.
	Note / Attention Observe the notes marked in this way to ensure correct function of the kit and to avoid operating errors for obtaining correct results.

The following systematic approach is introduced in the manual:

- The chapters and figures are numbered consecutively.
- A cross reference is indicated with an arrow (e.g. → “Notes on the use of this manual and the kit" p. 3).
- Working steps are numbered.

2 Safety precautions

NOTE
Read through this chapter carefully before to guarantee your own safety and a trouble-free operation.
Follow all the safety instructions explained in the manual, as well as all messages and information, which are shown.

All due care and attention should be exercised in handling the materials and reagents contained in the kit. Always wear gloves while handling these reagents and avoid any skin contact! In case of contact, flush eyes or skin with a large amount of water immediately.

FOR SINGLE USE ONLY!
This kit is made for single use only!

ATTENTION!
Don’t eat or drink components of the kit!
The kit shall only be handled by educated personnel in a laboratory environment!

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid any injuries. This kit could be used with potential infectious samples. Therefore, all liquid waste must be considered as potentially infectious and must be handled and discarded according to local safety regulation.

Please observe the federal, state and local safety and environmental regulations. Follow the usual precautions for applications using extracted nucleic acids. All materials and reagents used for DNA or RNA isolation should be free of DNases or RNases.

ATTENTION!
Do not add bleach or acidic components to the waste after sample preparation!

NOTE

Emergency medical information in English and German can be obtained 24 hours a day from:

Poison Information Center, Freiburg / Germany

Phone: +49 (0)761 19 240.

For more information on GHS classification and the Safety Data Sheet (SDS) please contact sds.innu@ist-ag.com.

3 Storage conditions

The kit is shipped at ambient temperature.

Upon arrival, store lyophilized and dissolved **Proteinase K** at 4 °C to 8 °C.

Store lyophilized **Carrier Mix** at -22 °C to -18 °C. It is recommended to divide dissolved **Carrier Mix** stock solution into aliquots for storage at -22 °C to -18 °C. Do not freeze and thaw **Carrier Mix** stock solution more than 3 times.

All other components of the innuPREP TCM DNA Extraction Kit should be stored dry, at room temperature (15 °C to 30 °C). When stored at room temperature, the kit is stable until the expiration date printed on the label on the kit box.

If there are any precipitates within the provided solutions solve these precipitates by careful warming. Before every use make sure that all components have room temperature.

4 Functional testing and technical assistance

The IST Innuscreen GmbH guarantees the correct function of the kit for applications as described in the manual. This product has been produced in an ISO 13485 certified facility.

We reserve the right to change or modify our products to enhance their performance and design. If you have any questions or problems regarding any aspects of the **innuPREP TCM DNA Extraction Kit** or other IST Innuscreen GmbH products, please do not hesitate to contact us. For technical support or further information in Germany please contact info.innu@ist-ag.com. For other countries please contact your local distributor.

5 Product use and warranty

The kit is not designed for the usage of other starting materials or other amounts of starting materials than those, referred to in the manual (→ “Product specifications” p. 9). Since the performance characteristics of our kits have not been validated for application described above, the user is responsible for the validation of the performance of IST Innuscreen GmbH kits using other protocols than those described below. IST Innuscreen GmbH kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA’ 88 regulations in the U.S. or equivalents in other countries.

All products sold by the IST Innuscreen GmbH are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

NOTE
The kit is for research use only!

6 Kit components

6.1 Components included in the kit

	Σ 50
REF	845-KS-3200050
Lysis Tube V	50
Lysis Solution MA	2 x 25 ml
Binding Solution RBS	10 ml
Carrier Mix	For 1 x 1.25 ml working solution
RNase-free Water	2 ml
Proteinase K	for 2 x 1.5 ml working solution
Precipitation Buffer P	6 ml
Prefilter	50
Spin Filter	2 x 50
Washing Solution C	30 ml
Washing Solution BS (conc.)	5 ml
Elution Buffer	10 ml
Receiver Tubes	10 x 50
Elution Tubes	2 x 50
Manual	1

6.2 Components not included in the kit

- 1.5 ml tubes
- 96–99.8 % ethanol, non-denatured or methylated
- 80 % ethanol, non-denatured or methylated
- ddH_2O for dissolving **Proteinase K**

7 Product specifications

1. Starting material:
   - Different kind of wood samples
   - Plants material
   - Different kinds of beans

2. Time for isolation:
   - Approximately 25 minutes after lysis step

3. Typical yield:
   - Depends on sample and amount of starting material

4. Typical ration A_{260}:A_{280}:
   - 1.7–2.0

8 Initial steps before starting

- Add 1.5 ml of ddH2O to each vial of Proteinase K, mix thoroughly and store as described above.
- Add 1.25 ml RNase-free Water to each vial of Carrier Mix and mix thoroughly by pipetting up and down. Store as described above.
- Add 45 ml of 96-99.8 % ethanol to the bottle Washing Solution BS **(conc.)**, mix thoroughly and always keep the bottle firmly closed!
- Heat thermal mixer or water bath at 60 °C.
- Centrifugation steps should be carried out at room temperature.
- Avoid freezing and thawing of starting material.

9 Usage of Carrier Mix

9.1 Storage conditions and handling

- Add dissolved Carrier Mix to Lysis Solution MA immediately.
- Unused Carrier Mix should be kept frozen at -22 °C to -18 °C.
- Do not freeze and thaw the Carrier Mix more than 3 times.
- Internal control DNA can be detected by real-time PCR using the corresponding assay, as shown in the following table:

Name	Amount	Order No.
innuDETECT Internal Control DNA/RNA Assay	100 rxn	845-ID-0008100

9.2 Preparation of Lysis Solution MA / Carrier Mix

1. Add 1.25 ml RNase-free Water to **Carrier Mix** tube.
2. Mix thoroughly by pipetting up and down!

   After the preparation of **Carrier Mix** stock solution prepare the mixture of **Lysis Solution MA / Carrier Mix** as described in the following tables:

Component	5 samples	10 samples	n samples
Lysis Solution MA	4.4 ml	8.8 ml	880 µl x sample
Carrier Mix	55 µl	110 µl	11 µl x sample
Final volume	4.46 ml	8.91 ml	891 µl x sample

10 Protocols

10.1 Protocol 1: Isolation of Nucleic Acids from 50 mg of different samples

NOTE
For the homogenization of samples it is recommended to use the Homogenizer “Speed Mill” from Analytik Jena GmbH.

1. Weight **50 mg** and cut the sample material in small pieces. Transfer the sample into **Lysis Tube V**. Homogenize the sample in the Speed Mill for 2 × 2 minutes.
2. Add **800 µl** of **Lysis Solution MA / Carrier Mix** and **50 µl Proteinase K** to the homogenized sample and mix vigorously by pulsed vortexing for 10 seconds.

   Incubate at 60 °C for 3 hours.

NOTE
We recommend to use a shaking platform (thermomixer, water bath or another rocking platform) for a continuous shaking of the sample. No shaking will reduce the lysis efficiency.

3. Add **100 µl Precipitation Buffer P** and mix vigorously by pulsed vortexing for 10 seconds. Centrifuge at 11,000 x g (~11,000 rpm) for 5 minutes.
4. Transfer **400 µl** of the clear supernatant onto a Prefilter located in a Receiver Tube and centrifuge the tube at 11,000 x g (~11,000 rpm) for 1 minute. Discard the Prefilter.

   Don’t discard the Receiver Tube with the filtrate!
5. Transfer the flow through in new tube.
6. Add **400 µl absolute ethanol** to the lysed sample (flow through from step 5), mix by pipetting up and down several times.

NOTE
It is important that the sample and the **absolute ethanol** are mixed vigorously to get a homogeneous solution.

7. Apply the sample to the Spin Filter located in a Receiver Tube. Close the cap and centrifuge at 16,000 x g for 2 minutes.

NOTE
If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time

Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.

8. Open the Spin Filter and add **500 µl Washing Solution C**, close the cap and centrifuge at 11,000 x g for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
9. Open the Spin Filter and add **650 µl Washing Solution BS**, close the cap and centrifuge at 11,000 x g for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
10. Open the Spin Filter and add **650 µl absolute ethanol**, close the cap and centrifuge at 11,000 x g for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
11. Centrifuge at maximum speed for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube.
12. Place the Spin Filter into an Elution Tube. Carefully open the cap of the Spin Filter and add **100 µl Elution Buffer**. Incubate at room temperature for 2 minutes. Centrifuge at 11,000 x g for 1 minute.

NOTE
The DNA can be eluted with a lower or a higher volume of **Elution Buffer** (depends on the expected yield of genomic DNA). Elution with lower volumes of **Elution Buffer** increases the final concentration of DNA. Store

the extracted DNA at 4 °C to 8 °C. For long time storage placing at -22 °C to -18 °C is recommended.

NOTE

If the eluate is coloured or contains impurities, a second binding step must be carried out as described in Protocol 2!

10.2 Protocol 2: Re-binding step to remove the impurities from eluate

1. Add **200 µl Binding Solution RBS** to the eluate. Mix by pipetting up and down several times.

   Avoid thereby formation of foam.

2. Apply the sample to the Spin Filter located in a Receiver Tube. Close the cap and centrifuge at 11,000 x g for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
3. Open the Spin Filter and add **500 µl 80 % ethanol**, close the cap and centrifuge at 11,000 x g for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
4. Open the Spin Filter and add **650 µl 80 % ethanol**, close the cap and centrifuge at 11,000 x g for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
5. Open the Spin Filter and add **650 µl 80 % ethanol**, close the cap and centrifuge at 11,000 x g for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
6. Centrifuge at maximum speed for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube.
7. Place the Spin Filter into an Elution Tube. Carefully open the cap of the Spin Filter and add **100 µl Elution Buffer**. Incubate at room temperature for 2 minutes. Centrifuge at 11,000 x g for 1 minute.

NOTE

Extracted DNA is pure and ready to use for downstream application.

11 Troubleshooting

Problem / probable cause	Comments and suggestions
Clogged Spin Filter	
Insufficient disruption or homogenization	Increase lysis time. Increase centrifugation speed. Reduce amount of starting material. Cut the starting material in small pieces and increase homogenization time.
Little or no DNA eluted	
Insufficient homogenization	Increase homogenization time and/or the speed of homogenization (using either homogenizer).
Insufficient lysis	Increase lysis time. Reduce amount of starting material. Overloading of Spin Filter reduces yield!
Incomplete elution	Prolong the incubation time with Elution Buffer to 5 minutes or repeat elution step once again. Take a higher volume of Elution Buffer.
Insufficient mixing with absolute ethanol	Mix sample with absolute ethanol by pipetting up and down several times prior to transfer of the sample onto the Spin Filter.
Low concentration of extracted DNA	
Too much Elution Buffer	Elute the DNA with lower volume of Elution Buffer.
Degraded or sheared DNA	
Old material insufficient	Old material often contains degraded DNA.
RNA contaminations of extracted DNA	
RNA contaminations of extracted DNA	Perform RNase digestion direct after precipitation step
Eluates are yellow	
Too much starting material and/or wood samples with strong colour pigments	Perform re-binding step as described in Protocol 2.

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