MBT109 InstaDNA™ Kit

Kit Contents

Kit contents provided	Product code	Packing	
		25 NO	100 NO
Hi705™ Micro Cards	MBT093	25 nos.	4X25 nos.
Proteinase K Solution (20 mg/ml)	DS0013	4 ml	15 ml
Wash Solution Concentrate (WS)	DS0012	50 ml	200 ml
TE Buffer	DS0086	130 ml	500 ml
Solution ID	DS0117	40 ml	140 ml
Solution ID1	DS0119	90 ml	360 ml
Solution ID2	DS0120	160 ml	2X310 ml
Sterile Ziplock pouches (for individual micro card storage)	-	25 nos.	4X25 nos.
Sterile Silica Gel Packs (for individual micro card storage)	-	50 nos.	4X50 nos.

HiMedia's InstaDNA™ Kit provides a rapid and cost-effective solution for extraction of DNA for Molecular Biology, Research, Clinical, Criminal and Forensic applications. The kit includes Hi705™ Micro Card and card elution buffer (Solution ID) required for instant DNA extraction.

It allows for instant extraction of ultrapure grade DNA from the card for various downstream applications like PCR etc.

Hi705™ Micro Card (MBT093)

Features:

- Non-treated Specimen matrix, no added chemistry
- The specimen dries and the cells will lyse. As the cell lyses, the DNA is released and unravels into the matrix.
- Long Term Ambient Storage of DNA, 21years+
- Proteins are stable for short term applications

ISO 9001-2008 CERTIFIED

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Strengths:

- Ambient Storage 21 years+ for nucleic acid applications
- Automatable for specimen handling systems
- No chemistry to interfere in processing
- Low cost compared to treated/coated cards
- Quantitative
- Proteins are stable for short term applications
- Fantastic for long term blood storage
- Only non chemically treated card available that has a indicating product - Ideal for short-term buccal applications

Weaknesses:

- Does not inactivate or kill the pathogens
- Proteins will only be active for a short time

Applications:

- Forensics
- Transgenic identification
- Transfusion medicine
- Plasmid screening
- Food and agriculture testing
- Drug discovery
- Genomics
- STR analysis
- Animal identification
- Whole genome amplification
- Molecular biology

Storage

The InstaDNA Kit is stable for 2 years at ambient temperature (15-30°C). The Hi705 cards can be stored at various temperatures; from ambient temperature (i.e. 15-30°C) all the way to -20°C.

Sample Protection and Handling

Always wear gloves to avoid contamination of Hi705 cards. Please follow your laboratories standard operating protocols and precautions when handling biological specimens.

Description

Hi705 cards provided in the InstaDNA Kit, are designed for room temperature collection, shipment, archiving of nucleic acids from variety of biological samples for PCR analysis. These include (but are not limited to) blood, buccal cells, tissue, cultured cells, microorganisms and plant tissue.

Use: To use Hi705 cards, simply apply sample (liquid or pressed tissue), air dry at room temperature, and then remove a small punch (the size of which needs to be determined by application). The punch can be used without washing for direct amplification. The Hi705 cards can also be prepared in the traditional method of sample preparation (see below).

NOTE: After sample application, store the Hi705 card in a sterile ziplock (provided) with two silica gel packs (provided) in each ziplock.

Materials needed but not provided

- Foam Tipped Swab (Product Code: PW1174) (for buccal protocol)
- Parafilm® (Product code: LA018) (for plant protocol)
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- Ethanol (96 - 100%)
- Molecular Biology Grade Water (Product code: ML024)
- Heating block
- Sterile forcep
- Sterile Microtubes
- Micropipettes & tips

Instructions:

Application of Blood Samples (fresh whole blood, or with certain anticoagulants):

1. Label the Hi705 card with the appropriate sample information and identification.
2. Drop the blood onto the card in a circular motion within the circle area. Avoid repeated dropping the blood in the same location of the liquid sample as it will overload the chemicals on the card. Also, do not rub or smear the blood onto the card.
3. Dried blood spots will appear darker than freshly spotted ones. Let the cards dry thoroughly prior to any sample punching or processing.
4. After drying completely, samples applied to the Hi705 cards are now ready either for immediate processing or archiving.
5. Drying time may vary due to humidity and conditions; please follow your laboratories standard operating procedures and guidelines.
6. Proceed to **Downstream DNA Analysis** (below).

Collection and transfer of buccal cell samples:

1. Place the Hi705 card on a clean, dry, flat surface. Label the card with appropriate sample information and identification.
2. Use a sterile Foam Tipped Swab (Product Code: PW1174) (not provided). Remove the protective packaging from the swab.
3. Hold the plastic handle of the swab, place the foam tip in the mouth and run the foam tip along the fold of the cheek, by the gums and under the tongue, collecting the cells from the sides of the walls of the mouth. Rub one side of the foam tip on the inside of the cheek for 15 seconds. Repeat using the opposite side of the foam tip for the other cheek. Remove the swab from the mouth.
4. Press the flat surface of the foam tip within the sample circle area. Without lifting the foam tip from the card, squeeze the tip using a side-to-side rocking motion, at least three times to completely saturate the collection circle area. Turn the swab over and repeat with the other side of the foam tip within the same circle.

   NOTE: Circle the area of the sample location with a ballpoint pen or pencil. If buccal cells are to be applied to more than one Hi705 circle area, use a new swab.

5. After drying completely, samples applied to the Hi705 cards are now ready either for immediate processing or archiving.

6. Drying time may vary due to humidity and conditions; please follow your laboratories standard operating procedures and guidelines.

NOTE: Other swabs can also be used, such as the Sterile Polyester Tipped Swab (Product Code: PW1180).

7. Proceed to **Downstream DNA Analysis** (below).

Application of Bacterial Samples (for bacterial genomic DNA) Bacterial colonies:

1. Concentration of sample may vary per sample type; please follow your laboratories standard operating procedures and guidelines.

2. Apply 5–10 µl of bacterial suspension to the Hi705 card and circle the area of application with a ballpoint pen or pencil.

3. After drying completely, samples applied to the cards are now ready either for immediate processing or archiving.

4. Drying time may vary due to humidity and conditions; please follow your laboratories standard operating procedures and guidelines

5. Proceed to **Downstream DNA Analysis** (below).

Collection of Tissue/Cell culture samples:

1. Tissue culture cells can be applied to Hi705 Cards at a concentration of >300 cells/µl for nucleic acid analysis.

2. After drying completely, samples applied to the Hi705 cards are now ready either for immediate processing or archiving.

3. Drying time may vary due to humidity and conditions; please follow your laboratories standard operating procedures and guidelines.

4. Proceed to **Downstream DNA Analysis** (below).

Collection of Plant samples:

Direct leaf press:

1. Place leaf material directly onto a circle of the Hi705 card. Place a piece of Parafilm® (Product code: LA018) (not provided) or similar plastic over the leaf.

2. Apply pressure &/or tap the leaf area with a blunt instrument such as a pestle or small hammer.

3. When the extract is seen through to the back of the Hi705 card, the collection process is finished.

4. After drying completely, samples applied to the Hi705 cards are now ready either for immediate processing or archiving.

5. Drying time may vary due to humidity and conditions please follow your laboratories standard operating procedures and guidelines.

6. Proceed to **Downstream DNA Analysis** (below).

Downstream DNA Analysis:

General Preparation Instructions:

Dilute Wash Solution Concentrate (WS) (DS0012) as follows:
Dilute Wash Solution Concentrate (WS) (DS0012) in the ratio 1:3 using ethanol (96-100 %) and mix thoroughly. For example, to 1ml of Wash Solution Concentrate (WS), add 3ml of ethanol (96-100%).

Number of Preps	Wash Solution Concentrate (WS)	Ethanol (96-100%)
25	50 ml	150 ml
100	200 ml	600 ml

1. Place punch or cutting of sample on the Hi705 card into the container.

2. The initial wash step requires five minute room temperature incubation in 150 µl distilled water per sample punch in a 200µl PCR tube. Remove the distilled water and discard.

3. Add 5µl of Proteinase K (20 mg/ml) (Product Code: DS0013) to 150 µl of diluted Wash Solution (WS) (Product Code: DS0012). Incubate sample punch for 30 minutes at 65°C. Discard the wash solution.

4. Add another 150 µl of diluted Wash Solution (WS) to the sample punch and incubate at room temperature for 3 minutes. Discard the wash solution and repeat for a total of two wash steps.

5. Add 200 µl of TE Buffer (Product Code: DS0086), incubate at room temperature for five minutes. Discard the rinse solution.

6. Dry the sample punch at 56-65°C until dry or overnight.

7. Proceed to PCR amplification according to the laboratory's Standard Operating Procedures for sample.

NOTE: After the card punch is processed, the user can either directly proceed to PCR amplification or can elute out the DNA from the card (for procedure, refer below) and then proceed to PCR amplification.

DNA ELUTION FROM CARD (OPTIONAL)

Elution protocol I

NOTE: The eluted DNA may not be stable for long term-storage. The user may follow Elution protocol II for the same.

1. To elute the DNA from card, place the processed card punch in a new sterile microtube (not provided) containing 50µl Solution ID (DS0117).
2. Incubate the tube at 95°C in a heating block for 5 minutes.

3. Using a sterile forcep, remove the card punch from the solution (Squeeze to recover maximum volume of the eluate). The solution containing DNA can be stored (2-8°C or at -20°C) for further analysis (viz. PCR).

Elution protocol II

1. To elute the DNA from card, place the processed card punch in a new sterile microtube (not provided) and add 140µl of Solution ID1 (DS0119) to the card.
2. Incubate at 65°C for 5 minutes.
3. Add 260µl of Solution ID2 (DS0120) and pulse vortex for 5 times to mix thoroughly.
4. Incubate the sample for 10 minutes at room temperature. Pulse vortex for 10 times to mix.
5. Using a sterile forcep, remove the card punch from the solution. The solution containing DNA can be stored (short-term storage at 2-8°C or long-term storage at -20°C) for further analysis (viz. PCR).

DNA Amplification Protocol

1. Specimen Punch

   a. Buccal: It is recommended that 1mm - 3mm punch or cutting size should be used for amplification. Place the cut specimen into the PCR mastermix.

   b. Blood: It is recommended that 1mm - 3mm punch or cutting size should be used for amplification. Place the cut specimen into the PCR mastermix.

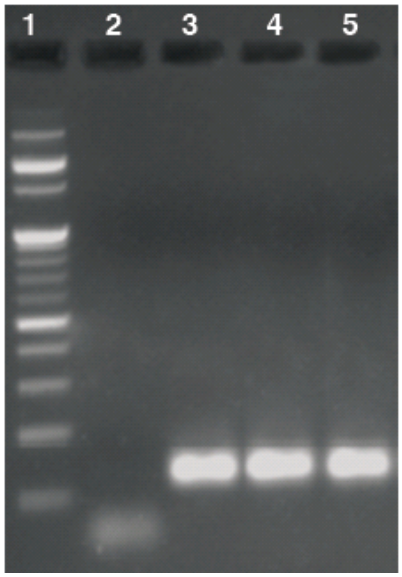
   NOTE: If the DNA in the sample is less concentrated, the user can cut a punch size of 5mm and then proceed to PCR amplification.

2. Complete reaction volumes should be used for PCR analysis.

3. Proceed to PCR direct amplification according to laboratory's Standard Operating Procedures for sample punches.

Please refer disclaimer Overleaf.

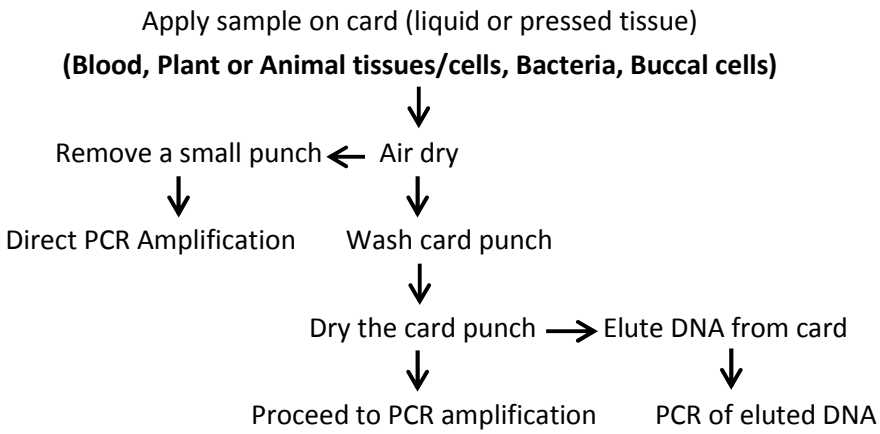
PCR Data from *M. tuberculosis* culture (H37RV) spotted onto Hi705™ Micro card using InstaDNA™ Kit



10µl of PCR product was loaded on 1% agarose gel

Lane 1: 100bp DNA Ladder
Lane 2: Negative Control
Lane 3: PCR data of *M. tuberculosis* (H37RV) DNA obtained using MB545
Lane 4: PCR data of *M. tuberculosis* culture (H37RV) spotted on Hi705™ Micro card (direct card punch PCR)
Lane 5: PCR data of *M. tuberculosis* culture (H37RV) spotted on Hi705™ Micro card (after DNA elution from card punch using InstaDNA™ Kit)

Flowchart for Instant DNA Extraction using InstaDNA™ Kit



Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.

Disclaimer :

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