Ver. 1902-00 (E)
FOR EXPORT ONLY

Punch-it™ NA-Sample Kit

Kit Contents

Punch-it™ NA-Sample Kit			
Cat. No.	**PINA15** **(15 preps/kit)**	**PINA50** **(50 preps/kit)**	**PINA200** **(200 preps/kit)**
Punch-it™ kit	15ea	50ea	50ea x 4ea
Puncher	15ea	50ea	50ea x 4ea
Lysis buffer	9.6 ml **(Add 6 ml isopropanol)**	32 ml **(Add 20 ml isopropanol)**	32 ml x 4ea **(Add 20 ml isopropanol)**
Washing buffer	1.8 ml **(Add 7.2 ml ethanol)**	6 ml **(Add 24 ml ethanol)**	6 ml x 4 ea **(Add 24 ml ethanol)**
Instruction for Use	1ea	1ea	1ea

※ **Store at room temperature**

Description

Punch-it™ NA-Sample Kit is a nucleic acid purification kit designed for instant isolation of total nucleic acids (DNA and RNA) from a little amount of various samples such as animals, blood, plants, bacteria and viruses. Using the paper chromatographic method, enzyme inhibitors are efficiently removed from the samples by this kit. The purified nucleic acids bound on membrane could be used directly in PCR or RT-PCR reactions as templates.

Applications

Purification of nucleic acid for PCR or RT-PCR
Purification of nucleic acid for molecular diagnostics

Quality control

Punch-it™ NA-Sample Kit was tested for the isolation of total nucleic acids from various samples such as animals, blood, plants, bacteria and viruses. Nucleic acid purified with Punch-it™ NA-Sample Kit was evaluated by the amplification of target DNA or transcript using PCR or RT-PCR.

Important thing to do before starting

Before using **Lysis Buffer**, **add isopropanol according to the bottle label** to obtain a working solution.

Before using **Washing Buffer**, **add absolute ethanol according to the bottle label** to obtain a working solution, You may use 80% ethanol, instead of Washing Buffer.

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Protocol

Sample Preparation

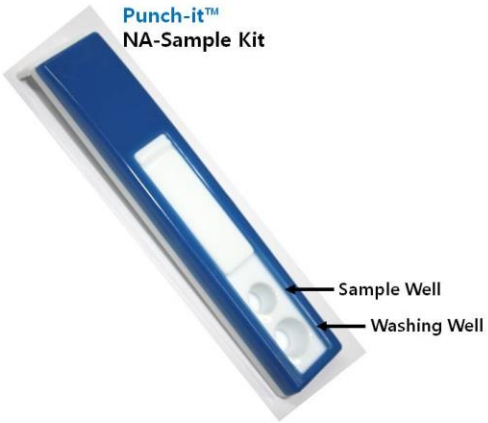
[Whole Blood, saliva or purified virus]

1) Prepare **50 µl of Lysis Buffer** into a 1.5 ml microcentrifuge tube (not provided).

2) Add **5 ~ 10 µl of sample** into the tube including lysis buffer and mix by tapping.
   ※ In case of blood sample, Heparin, EDTA or citrate-treated whole blood are all suitable for this kit.

3) Transfer the lysate **into the Sample Well** of this kit,
   and wait until the sample solution is completely absorbed into the membrane.
   ※ The amount of loading on the sample well should not exceeds 60 µl.

4) Go to 「**2. Common step**」.



[Animal tissue, plant leaves or fruit]

1) Prepare **100 µl of Lysis Buffer** into a 1.5 ml microcentrifuge tube (not provided).

2) Take a small piece of tissue (about ~5 mm diameter size) from animal or plant tissue.

3) Add tissue sample into the Lysis Buffer aliquot and crush the sample with a 100 µl pipette tip
   by pressing it briefly against the tube wall.
   ※ If larger amount of tissue sample is used (>5mm), increase the volume of the lysis buffer to 200 µl.

4) Incubate at RT for 2 min for tissue lysis and nucleic acid releasing.

5) Spin briefly and transfer **50 µl of the supernatant** of lysate **into the Sample Well** of this kit,
   and wait until the sample solution is completely absorbed into the membrane.

6) Go to 「**2. Common step**」.

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[Plant seeds]

1) Plant seeds crush finely using a hammer, bead beater or tissuelyser.

2) Transfer crushed seed (about 10~20 mg) to 1.5 ml microcentrifuge tube (not provided).

3) Add **300 µl of Lysis Buffer** and briefly mix by vortexing for 15 second.
※ If larger amount of seeds is used (>20mg), increase the volume of the lysis buffer to 500 µl.

3) Incubate at RT for 2 min for sample lysis and nucleic acid releasing.

4) Spin briefly and transfer **50 µl of the supernatant** of lysate **into the Sample Well** of this kit,
and wait until the sample solution is completely absorbed into the membrane.

5) Go to 「**2. Common step**」.

[Buccal swab]

1) Prepare **300 µl of Lysis Buffer** into a 1.5 ml microcentrifuge tube (not provided).

2) Cut the cotton tip where the cells were collected.

3) Add cotton tip into the lysis buffer aliquot and briefly mix by vortexing or tapping.

4) Spin briefly and transfer **50 µl of the supernatant into the Sample Well** of this kit,
and wait until the sample solution is completely absorbed into the membrane.

5) Go to 「**2. Common step**」.

[Urine]

1) Prepare 1 ml of sample into a 1.5 ml microcentrifuge tube (not provided),
and centrifuge at 12,000 rpm for 1 min.

2) Remove supernatant by pipetting.

3) Add **50 µl of Lysis Buffer** and mix by vortexing vigorously until the pellet resuspended completely.

4) Transfer lysate **into the Sample Well** of this kit,
and wait until the sample solution is completely absorbed into the membrane.

5) Go to 「**2. Common step**」.

[Soil or stool]

1) Prepare **10~20 mg of sample** into a 1.5 ml microcentrifuge tube (not provided).

2) Add **500 µl of Lysis Buffer** and mix by vortexing vigorously for 15 second.
   ※ **If larger amount of sample is used (>20 mg), increase the volume of the lysis buffer to 1 ml.**

3) Centrifuge at 12,000 rpm for 1 min.

4) Transfer **50 µl of the supernatant into the Sample Well** of this kit
   and wait until the sample solution is completely absorbed into the membrane.

5) Go to 「**2. Common step**」.

2. Common step (After loading lysate to Sample Well)

1) Load **200 µl of Washing Buffer (80% ethanol) into the Washing Well** and stay for 3~5 min.
   ※ **If the sample well is not clear after treatment of washing buffer, repeat this step with additional loading of 100 µl of Washing buffer.**
   ※ **Even if the membrane on Sample Well is not completely clean after this washing step, PCR and RT-PCR reactions are not affected significantly.**

2) **Punch out about 1 mm size of membrane from the bottom of Sample Well** using a puncher
   or tip, and directly apply in PCR or RT-PCR reactions as template.
   ※ **Even if the membrane piece is not completely dried, PCR and RT-PCR reactions are not affected significantly.**
   ※ **The nucleic acids on the kits are stable for 6 months at 4℃.**

<Recommendation for PCR or RT-PCR>

a) Optimal reaction volume for PCR or RT-PCR is 50 µl.
b) Optimal cycle number is 35 ~ 45 cycles.
c) Do not use high-fidelity PCR enzymes such as *Pfu* DNA polymerase or their derivatives.

Products

Cat. No.	Products	Size
PINA15	Punch-it™ NA-Sample Kit	15 Preps
PINA50	Punch-it™ NA-Sample Kit	50 Preps
PINA200	Punch-it™ NA-Sample Kit	200 Preps

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