G-spin™ Total DNA Extraction Kit

The Instruction Manual for Genomic DNA Extraction from Cultivated animal cells, tissues, Gram negative bacteria and Blood using silica membrane.

RUO *Research Use Only* REF *S17045* Σ 4

DESCRIPTION

- G-spin™ Total DNA Extraction Mini Kit provide fast and easy methods for purification of total DNA from cultured animal cell, animal tissue, rodent tail, fixed tissue, animal hair, gram negative bacteria, and blood samples for reliable PCR and Southern blotting. Furthermore, we have tested G-spin™ Total DNA Extraction Mini Kit to get more practical data with a lot numbers of biological samples.
- The simple G-spin™ Total DNA Extraction procedures, which are ideal for simultaneous processing of multiple samples, yield pure DNA ready for direct amplification in just 20 ~ 30 minutes. The G-spin™ Total DNA Extraction Mini Kit procedure is suitable for use with fresh or frozen whole blood and blood which has been treated with citrate, heparin, or EDTA. Pre-separation of leukocytes is not necessary.
- Purification requires no phenol/chloroform extraction or alcohol precipitation, and involves very little handling. DNA is eluted in Buffer CE, TE (10:1), 10mM Tris (pH 7.5 ~ 8) or water, ready for direct addition to PCR or other enzymatic reactions. Alternatively, it can be safely stored at –20°C for later use. The purified DNA is free of protein, nucleases, and other contaminants or inhibitors. DNA purified using G-spin™ Total DNA Extraction Mini Kit is up to 50 kb in size, with fragments of approximately 20–30 kb predominating. DNA of this length denatures completely during thermal cycling and can be amplified with high efficiency.
- G-spin™ Total DNA Extraction Mini Kit provides various protocols. You can also extract genomic DNA from various your biological samples by selecting an appropriate protocol from Protocol list (see Table 1). If you need some more information in selecting a protocol, please do not hesitate to contact our Technical Assistance Team.

CHARACTERISTICS

- **Speed** : Takes only 20 ~ 30 minutes to extract genomic DNA.
- **Smart** : High quality and quantity of DNA recovery
- **Steady** : Complete removal of inhibitors and contaminants for accurate down stream applications. And the freeze-dried formulated enzyme has been improved DNA extraction stability.
- **Stage-up** : No need various DNA Extraction kit – vast applicability. The Kit is suitable to use various kinds of biological samples. Advanced GxN technology for rapid and efficient purification of DNA without ethanol precipitation.

KIT CONTENTS

Label		Contents
Buffer CL		0.8 ml
Buffer BL[1]		0.8 ml
Buffer WA[1]		2.8 ml
Buffer WB		2.8 ml
Buffer CE[2]		0.5 ml
Spin Column[3] / Collection Tube[4]		4 ea
RNase A (Lyophilized powder)[5]	Add 25µl of DW	0.25mg
Proteinase K (Lyophilized powder)[5]	Add 88µl of DW	1.76 mg

1. This buffer contains chaotropic salt.
2. DNase / RNase free Ultra-Pure solution.
3. The Columns contain silica membrane
4. Polypropylene tube for 2ml volume
5. The lyophilized RNase A and Proteinase K can be stored at room temperature (15–25°C) until the kit expiration date without affecting performance. The lyophilized RNase A and Proteinase K can only be dissolved in D.W.; dissolved RNase A and Proteinase K should be immediately stored at -20℃. These solutions are stable at -20°C for up to 24 months and 20 times frozen-thawing until the kit expiration date.

STORAGE

G-spin™ Total DNA Extraction Kit should be stored dry at room temperature (15–25°C). Under these conditions, G-spin™ Total DNA Extraction Kit can be stored for up to 24 months without showing any reduction in performance and quality. The lyophilized RNase A and Proteinase K can be stored at room temperature (15–25°C) until the kit expiration date without affecting performance. The lyophilized RNase A and Proteinase K can only be dissolved in D.W.; dissolved RNase A and Proteinase K should be immediately stored at -20℃. These solutions are stable at -20°C for up to 24 months and 20 times frozen-thawing until the kit expiration date.

APPLICATIONS

- Cancer research
- Human genetic research
- Detection Assay : PCR, real time PCR
- DNA hybridization : Southern blotting, microarray
- Gram negative bacterial research
- Viral DNA Research

PROTOCOL LIST

Table 1. Protocols according to the Blood Sample Groups (8 Protocols)

Samples	Protocol Type
Blood, Body Fluids	Type A Protocol
Tissues , Rodent tail	Type B Protocol
Cell, Buffy coat, Bacteria	Type C Protocol
Dried Blood Spots	Type D Protocol
Fixed Tissues	Type E Protocol
Bacteria	Type F Protocol
Biological swabs	Type G Protocol
Animal Hair	Type H Protocol

NOTICE BEFORE USE

G-spin™ Total DNA Extraction Kit provides almost all reagents for extracting DNA, including RNase A and Proteinase K. However, you should prepare some equipments and reagents as follows for a fast and easy extraction. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

• Common equipment and reagents

- Equipment for disruption and homogenization, mechanical tissue grinder like pestle
- Pipettes and pipette tips
- Vortex mixer
- Microcentrifuge tubes (1.5 ml)
- Absolute ethanol (EtOH, 96~100%)
- Ice
- 1X PBS Buffer
- Water bath or heating block
- Microcentrifuge with rotor for 2.0 ml tubes
- Liquid nitrogen
- 80% ethanol
- Other general lab equipments
- Xylene Solution (for paraffin block)

SAFETY INFORMATION

All chemicals should be considered as potentially hazardous. When working with chemicals, always should wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please request the appropriate material safety data sheets (MSDS). Do not add bleach or acidic solutions directly to the waste. Buffer BL and Buffer WA contains a chaotropic salts, which can form highly reactive compounds when combined with bleach. If the liquid spill contains potentially infectious agents, clean the affected area first with laboratory detergent and water, then with a suitable laboratory disinfectant. Only persons trained in laboratory techniques and familiar with the principles of good laboratory practice should handle these products.

CAUTION:
DO NOT add bleach or acidic solutions directly to the sample preparation waste.

QUALITY CONTROL

- In accordance with iNtRON's ISO-certified Total Quality Management System, each lot of G-spin™ Total DNA Extraction Kit is tested against predetermined specifications to ensure consistent product quality. The quality of the isolated genomic DNA was checked by restriction analysis, agarose gel electrophoresis, and spectrophotometric determination.
- G-spin™ column control : The DNA binding capacity was tested by determining the recovery with 10 ~ 15 μg of genomic DNA from 1 x 10^6 cultivated cells.
- RNase A / Proteinase K : In case of RNase A, the activity was determined 20K ~ 25K unit per mg of protein using tolura yeast RNA hydration test. Also, in case of Proteinase K, the activity was determined from cleavage of the substrate releasing p-nitroaniline which can be measured spectrophotometrically at 410nm.
- Buffer control : Conductivity and pH of buffers were tested and found to be within the pre-determinated ranges descrbed below

Buffer	Conductivity	pH
Buffer CL	13.5 ~ 15.5 mS/cm	7.6 ~ 8.3
Buffer BL	120 ~ 140 mS/cm	6.9 ~ 7.6
Buffer WA	28 ~ 36 mS/cm	6.9 ~ 7.7
Buffer WB	10 ~ 12 mS/cm	7.4 ~ 8.0
Buffer CE	550 ~ 700 μS/cm	7.4 ~ 8.0

PRODUCT WARRANTY AND SATISFACTION GUARANTEE

All products undergo extensive quality control test and are warranted to perform as described when used correctly. Immediately any problems should be reported. Satisfaction guarantee is conditional upon the customer providing full details of the problem to iNtRON within 60 days, and returning the product to iNtRON for examination.

PRODUCT USE LIMITATIONS

The G-spin™ Total DNA Extraction Kit is intended for research use only. Prior to using it for other purposes, the user must validate the system in compliance with the applicable law, directives, and regulations.

G-spin™ Total DNA Extraction Kit is developed, designed, and sold for research purpose only. They are not to be used for human or animal diagnosis of diseases. Do not use internally or externally in humans or animals. Be careful in the handling of the products.

COLUMN INFORMATION

- The G-spin™ Total DNA Extraction Kit Spin Column

Column membrane[1]	Silica-based membrane
Spin Column[1]	Individually, in inserted in a 2.0 ml Collection Tube[2]
Loading Volume	Maximum 800 μl
DNA Binding Capacity	Maximum 45 μg
Recovery	85 - 95% depending on the elution volume
Elution Volume	Generally, eluted with 30 – 200 μl of elution buffer

[1] Do not store the Column packs under completely dried conditions. It may be affected to DNA binding capacity. The Spin Columns are stable for over 2 year under these conditions

[2] Additional Collection Tubes (100 ea) are also supplied for your convenient handling.

IMPORTANT POINTS BEFORE STARTING

- **Lyophilized RNase A** : Dissolve the RNase A in 25 μl of pure D.W. to each vial. The lyophilized RNase A can be stored at room temperature (15–25°C) until the expiration date without affecting performance. The lyophilized RNase A can only be dissolved in D.W.; dissolved RNase A should be immediately stored at -20℃. The RNase A solution is stable at -20°C for up to 24 months and 20 times frozen-thawing until the kit expiration date
- **Lyophilized Proteinase K :** Dissolve the Proteinase K in 88 μl of pure D.W. to each vial. The lyophilized Proteinase K can be stored at room temperature (15–25°C) until the expiration date without affecting performance. The lyophilized Proteinase K can only be dissolved in D.W.; dissolved Proteinase K should be immediately stored at -20℃. The Proteinase K solution is stable at -20°C for up to 24 months and 20 times frozen-thawing until the kit expiration date
- Preheat a water bath or heating block
- Equilibrate Buffer IE or distilled water for elution to room temperature.
- If Buffer CL or Buffer BL contains precipitates, dissolve by heating to 70°C with gentle agitation.
- Centrifugation : All centrifugation steps are carried out at RT (15 - 25°C)

SAMPLE PREPARATION

◈ Amounts of starting material

Use the amounts of starting material indicated in Table 2.

Table 2. Amounts of starting material for G-spin™ Total Kit procedures

Sample	Amount
Blood, plasma, serum	200 μl
Buffy coat	200 μl
Tissue	25 mg*
Cultured cells	1 x 10^6 cells
Bacterial culture (Liquid Culture)	3 OD (OD_{600})

* When isolating DNA from spleen, 10 mg samples should be used.

Small samples should be adjusted to 200 μl with PBS before loading. For samples larger than 200 μl, the amount of Buffer CL and other reagents added to the sample before loading must be increased proportionally. Application of the lysed sample to the spin column will require more than one loading step if the initial sample volume is increased. The amounts of Buffer WA and Buffer WB used in the wash steps do not need to be increased. Scaling up the tissue protocol is possible in principle. The volumes of Buffer CL and proteinase K used should be increased proportionally, while the volumes of wash and elution buffers should remain constant. The user should determine the maximum amount of tissue used. It is important not to overload the column, as this can lead to significantly lower yields than expected.

◈ Preparation of buffy coat

Buffy coat is a leukocyte-enriched fraction of whole blood. Preparing a buffy-coat fraction from whole blood is simple and yields approximately 5–10 times more DNA than an equivalent volume of whole blood.

Prepare buffy coat by centrifuging whole blood at 2500 x g for 10 minutes at room temperature. After centrifugation, 3 different fractions are distinguishable: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes; and the bottom layer contains concentrated erythrocytes.

◈ Copurification of RNA

G-spin™ Total Kit spin columns are able to copurify DNA and RNA when both are present in the sample (see Table 3). RNA may inhibit some downstream enzymatic reactions but will not inhibit PCR. If RNA contained genomic DNA is required, the the treatment of RNase A should be bypassed to the sample.

Table 3. Amounts of starting material for G-spin™ Total Kit procedures

Sample	DNA composition	RNA composition
Blood, plasma, serum, Buffy coat	95~100 %	0~5%
Tissues (Liver, Heart / Others)	15~20% / 40~50%	80~85%/50~60%
Cultured cells, Bacterial culture	30~50%	50~80%

PROTOCOL A (for Blood, body fluids)

1. Pipet 200 µl of whole blood or body fluids into a 1.5 ml microcentrifuge tube (not provided).
   Note : If the volume of sample is less than 200 µl, use Buffer CL or PBS Buffer
2. Add 20 µl of Proteinase K and 5 µl of RNase A Solution into sample tube and gently mix.
   Note : It is possible to add Proteinase K to blood sample that have already been measured into 1.5 ml tube. It is important to assure proper mixing after adding the Proteinase K and RNase A solution.
3. Add 200 µl of Buffer BL into upper sample tube and mix thoroughly.
   Note : Avoid any vigorous vortexing because doing so may induce genomic DNA breakage. In order to assure efficient lysis, it is important that the blood sample and Buffer BL are mixed thoroughly to yield a lysis solution.
4. Incubate the lysate at 56°C for 10 min.
   Note : For complete lysis, mix 3 or 4 times during incubation by inverting tube. If it lysis perfectly, the red color of lysate becomes the dark green.
5. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the rid.
6. Add 200 µl of absolute ethanol into the lysate, and mix well by gently inverting 5 - 6 times or by pipetting. DO NOT vortex. After mixing, briefly centrifuge the 1.5 ml tube to remove drops from inside of the lid.
   Note : This step is an equilibration step for binding genomic DNA to column membrane. It is important to assure proper mixing after adding the ethanol, until not showing 2-phase which is not mixed. Also, this step conduces to pass efficiently cell lysate through a column.
7. Carefully apply the mixture from step 7 to the Spin Column (in a 2 ml Collection Tube) without wetting the rim, close the cap, and centrifuge at 13,000 rpm for 1 min. Discard the filtrate and place the Spin Column in a new 2 ml Collection Tube (additionally supplied).
   Note : Close each Spin Column in order to avoid aerosol formation during centrifugation. Do not transfer any solid materials.
8. Add 700 µl of Buffer WA to the Spin Column without wetting the rim, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and reuse the Collection Tube.
9. Add 700 µl of Buffer WB to the Spin Column without wetting the rim, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and place the Column into a new 2.0 ml Collection Tube (additionally supplied), Then again centrifuge for additionally 1 min to dry the membrane. Discard the flow-through and Collection Tube altogether.
   Note : It is very important to dry the membrane of the Spin Column since residual ethanol may inhibit subsequent reactions. Following the centrifugation, remove carefully the Spin Column from the Collection Tube without contacting with the flow-through, since this will result in carryover of ethanol.
10. Place the Spin Column into a new 1.5 ml tube (not supplied), and 30 - 100 µl of Buffer CE directly onto the membrane. Incubate for 1 min at room temperature and then centrifuge for 1 min at 13,000 rpm to elute.
    Note : Elution with 30 µl (instead of 50 µl) increases the final DNA concentration, but reduces overall DNA yield conventionally.
    Note : A new 1.5 ml tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, the tube can be reused for the second elution step to combine the eluates.

PROTOCOL B (for Tissue, Rodent tail)

1. Take out the target organ from laboratory animal.
   Note : The fresh animal tissue can be used directly to isolation of genomic DNA. But if the tissues are not used immediately, those should be stored with liquid nitrogen (below -196℃) or deep freezer (below - 80℃) for long-term.
2. Slice off the prepared sample to suitable size by the scalpel or scissor.
   Note : To reduce disruption and homogenization time, we recommend to slice off it. In case of enzymatic sample lysis, cut the sample 0.6 ~ 1.2 cm (mouse) or 0.3 ~ 0.6 cm (Rat) length, then slice the sample into pieces as small as possible.
3. Place the sliced sample material into a grinding jar (mortar). Add liquid nitrogen to the mortar and freeze. Keep the sample submerged in liquid nitrogen, and disrupt carefully until the sample is homogenized completely. Allow the liquid nitrogen to evaporate, and proceed immediately to step 4.
   Note : Disruption and homogenization time depends on the tissue samples. We recommend to be disrupted completely until no tissue clumps are not visible. Clumps of tissue sample will be difficult to lyse properly and will result in a lower yield of DNA. It's very important to keep the sample frozen in liquid nitrogen during disruption and homogenization step to inhibit low DNA yields and degraded DNA. Be careful to handle liquid nitrogen.
4. Measure 25 mg of ground tissue sample, and then transfer into 1.5 ml tube using a spatula.
   Note : In order to prevent from thawing the frozen sample during transfer it, use pre-chilled the spatula and 1.5ml tube (When pre-chill the tube, the lid of tube MUST always be OPEN) with liquid nitrogen. The freeze-thaw repetition of frozen sample will result in the DNA degradation. And more, exceeding the recommended optimal amount of starting material will result in inefficient lysis, resulting in low DNA yield and purity. Ensure that the account of starting material is used, if the genomic DNA is prepared from spleen and thymus tissue, no more 10 mg should be used.
5. Add 200 µl Buffer CL, 20 µl Proteinase K and 5 µl RNase A Solution into sample tube and mix by vortexing vigorously.
   Note : Be sure that Proteinase K and RNase A solutions are always kept under freezer (below -10℃). In case of transcriptionally active cultured cell, contain large amount of RNA which will be co-purified with genomic DNA. RNA may inhibit downstream enzymatic reaction, but will not affect PCR.
6. Incubate the lysate at 56℃ (preheated heat block or water bath) for 10 ~ 30 min.
   Note : To help lysis tissue sample, mix the tube by inverting every 2 min during the incubation. Lysis time varies depending on the type of sample. However G-spin Total DNA Extraction Kit provides strong lysis mechanism against tissue sample. In case of cultured cell, it is enough to lysis completely for 10 ~ 15 mins, respectively. After incubation the lysate may appear viscous, but should not be gelatinous as it may clog the spin column.
7. After lysis completely, add 200 µl of Buffer BL into upper sample tube and mix thoroughly. Then incubate the mixture at 70℃ for 5min.
   Note : Avoid any vigorous vortexing because doing so many induce genomic DNA breakage. In order to assure efficient lysis, it is important that the lysate sample and Buffer BL are mixed thoroughly.
8. Centrifuge the sample tube at 13,000 rpm for 5 min to remove un-lysed tissue particles. Then carefully transfer 350 ~ 400 µl of the supernatant into a new 1.5 ml tube (not provided).
   Note : If insoluble tissue clumps remains in homogenated mixture, it will be occurred spin column clogging, sometimes. This step helps sample mixing with buffer during binding step by and large. Also It prevents column clogging from insoluble clumps.
9. Follow the Protocol A (for Blood, body fluids) from Step 6.

PROTOCOL C (Cell, Buffy coat)

1. Prepare the sample according to 1a or 1b.
   1a. Cells grown in suspension ; Transfer the culture fluid into 15 ml or 50 ml of centrifuge tube and pellet the culture by centrifugation for 5 min at 3,000 rpm. Remove the supernatant completely and wash the pellet with PBS or fresh media. Then resuspend the washed cell pellet in appropriate volume of PBS or fresh media.
   1b. Cells grown in monolayer ; Cells grown in monolayer can be detached from culture flask (or plate) by either 1) Trypsinization or 2) Using a cell scraper.
   1) To Trypsinize cells : Remove the medium and wash the cells with preheated (at 37℃) PBS. Then aspirate the PBS and add trypsin solution. After cells have become detached from culture flask (or dish), collect and wash the cells with PBS, then resuspend the washed cell pellet in appropriate volume of PBS or fresh media.
   2) Using a cell scrape, detach cells from culture flask or dish. Collect and wash the cells with PBS, then resuspend the washed cell pellet in appropriate volume of PBS or fresh media.
2. Determinate the cell number using cell counter (eg. hemocytometer) and transfer the appropriated number of cells (1 ~ 3 x 10^6 cells) to a new 1.5 ml microcentrifuge tube.
3. Pellet the cell by centrifugation for 1 min at 13,000 rpm and discard the supernatant. Resuspend completely the cell pellet with remnant supernatant by tapping or vigorously vortexing.
   Note : In order to ensure efficient lysis, it is essential that the cell pellet and remnant supernatant are mixed thoroughly to yield a homogeneous solution.
4. Add 200 µl Buffer CL, 20 µl Proteinase K and 5 µl RNase A Solution into sample tube and mix by vortexing vigorously.
   Note : Be sure that Proteinase K and RNase A solutions are always kept under freezer (below -10℃). In case of transcriptionally active cultured cell, contain large amount of RNA which will be copurified with genomic DNA. RNA may inhibit downstream enzymatic reaction but will not affect PCR.
5. Incubate the lysate at 56℃ (preheated heat block or water bath) for 10 ~ 30 min.
   Note : To help lysis tissue sample, mix the tube by inverting every 2 min during the incubation. Lysis time varies depending on the type of sample. In case of cultured cell, it is enough to lysis completely for 10 ~ 15 min, respectively.
6. After lysis completely, add 200 µl of Buffer BL into upper sample tube and mix throughly. Then incubate the mixture at 70℃ for 5min.
   Note : Avoid any vigorous vortexing because doing so many induce genomic DNA breakage. In order to assure efficient lysis, it is important that the lysate sample and Buffer BL are mixed thoroughly.
7. Follow the Protocol A (for Blood, body fluids) from Step 6.

Maxime PCR PreMix Kit (*i*-StarTaq)

for 20μl / 50μl rxn

Cat. No. 25165(for 20μl rxn, 96 tubes) **Cat. No. 25167**(for 20μl rxn, 480 tubes)
Cat. No. 25180(for 50μl rxn, 96 tubes)

DESCRIPTION

iNtRON's *Maxime* PCR PreMix Kit has not only various kinds of PreMix Kit according to experience purpose, but also a 2X Master mix solution.
Hot start PCR technique was developed as a method to minimize the deleterious effects of mispriming at lower temperatures during PCR. In a PCR reaction, even short incubations at temperatures below the optimum annealing temperature for a particular set of primers can result in mispriming, elongation and the subsequent formation of spurious bands.
Maxime PCR PreMix Kit (*i*-StarTaq) is the product what is mixed every component : *i*-StarTaq™ DNA Polymerase, dNTP mixture, reaction buffer, and so on- in one tube for 1 rxn PCR. This is the product that can get the best result with the most convenience system. The first reason is that it has every components for PCR, so we can do PCR just add a template DNA, primer set, and D.W.. The second reason is that it has Gel loading buffer to do electrophoresis, so we can do gel loading without any treatment. In addition, each batches are checked by a thorough Q.C., so its reappearance is high. It is suitable for various sample's experience by fast and simple using method.

STORAGE

Store at -20°C; under this condition, it is stable for at least a year.

CHARACTERISTICS

- Sensitivity : reduced or no amplification of non-specific products resulting from mispriming during PCR.
- Specificity : generating fragments of high specificity and high yield.
- Flexibility : available for various DNA template including cloned fragment, phage DNA, mammalian genomic DNA and etc.
- Ready to use: only template and primers are needed
- Stable for over 1 year at -20℃
- Time-saving and cost-effective

CONTENTS

- *Maxime* PCR PreMix(*i*-StarTaq, for 20μl rxn) 96 (480) tubes
- *Maxime* PCR PreMix(*i*-StarTaq, for 50μl rxn) 96 tubes

Component in	20 μl reaction	50 μl reaction
i-StarTaq™ DNA Polymerase	2.5U	5U
dNTPs	2.5mM each	2.5mM each
Reaction Buffer(10x)	1x	1x
Gel Loading buffer	1x	1x

PROTOCOL

1. Add template DNA and primers into *Maxime* PCR PreMix tubes(*i*-StarTaq).
   Note 1 : Recommended volume of template and primer : 3μl~9μl
   Appropriate amounts of DNA template samples
   - cDNA : 0.5-10% of first RT reaction volume
   - Plasmid DNA : 10pg-100ng
   - Genomic DNA : 0.1-1ug for single copy

   Note 2 : Appropriate amounts of primers
   - Primer : 5-20pmol/μl each (sense and anti-sense)
2. Add distilled water into the tubes to a total volume of 20μl or 50μ.
   Do not calculate the dried components

Example **Total 20μl or 50μl reaction volume**

PCR reaction mixture	Add	Add
Template DNA	1 ~ 2μl	2 ~ 4μl
Primer (F : 10pmol/μl)	1μl	2 ~ 2.5μl
Primer (R : 10pmol/μl)	1μl	2 ~ 2.5μl
Distilled Water	16 ~ 17μl	44 ~ 41μl
Total reaction volume	**20 μl**	**50 μl**

Note : This example serves as a guideline for PCR amplification. Optimal reaction conditions such as amount of template DNA and amount of primer, may vary and must be individually determined.

3. Dissolve the blue pellet by pipetting.
   Note : If the mixture lets stand at RT for 1-2min after adding water, the pellet is easily dissolved.
4. (Option) Add mineral oil.
   Note : This step is unnecessary when using a thermal cycler that employs a top heating method(general methods).
5. Perform PCR of samples.
6. Load samples on agarose gel without adding a loading-dye buffer and perform electrophoresis.

SUGGESTED CYCLING PARAMETERS

PCR cycle		Temp.	PCR product size		
			100-500bp	500-1000bp	1Kb-5Kb
Initial denaturation		94℃	2min	2min	2min
30-40 Cycles	Denaturation	94℃	20sec	20sec	20sec
	Annealing	50-65℃	10sec	10sec	20sec
	Extension	65-72℃	20-30sec	40-50sec	1min/Kb
Final extension		72 ℃	Optional. Normally, 2-5min		

Note : The PCR process is covered by patents issued and applicable in certain countries. iNtRON Biotechnology does not encourage or support the unauthorized or unlicensed use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.

EXPERIMENTAL INFORMATION

• Comparison with i-StarTaq™ and Maxime PCR PreMix(i-StarTaq™)

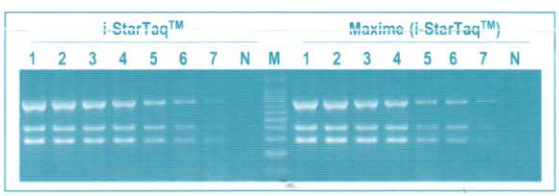

Fig.1. Comparison with *i*-StarTaq™ DNA polymerase and *Maxime* PCR PreMix (*i*-StarTaq™)
Comparison with *i*-StarTaq™ DNA Polymerase and *Maxime* PCR PreMix (*i*-StarTaq) by amplifying fyuA (780bp), tsh (420bp) and Irp2 (280bp) from variable amounts of *E.coli* gDNA.
Lane M, SiZer-100 DNA Marker; **lane 1,** 50 ng gDNA; **lane 2,** 10 ng gDNA; **lane 3,** 2 ng gDNA; **lane 4,** 400 pg gDNA; **lane 5,** 80 pg gDNA; **lane N**, Negative control

• Comparison with different company Kit

Fig.2. Comparison of *Maxime* PCR PreMix(*i-StarTaq*) and Supplier A's Hot-start PreMix system by amplifying 218 bp DNA fragment.
Total genomic DNA from cultivated human cell (K-562) was used as PCR template from 100 ng to 1.56 ng.
Lane M, SiZer-100 DNA Marker; **lane 1,** 100 ng; **lane 2,** 50 ng; **lane 3,** 25 ng; **lane 4,** 12.5 ng; **lane 5,** 6.25 ng; **lane 6,** 3.125 ng; **lane 7,** 1.56 ng; **lane N,** Negative control

iNtRON BIOTECHNOLOGY
www.intronbio.com / info@intronbio.com
T. (0505)550-5600 / F. (0505)550-5660