COSMO BIO CO., LTD.
Inspiration for Life Science

TOYOBO

Nucleic Acid Purification Kit

MagExtractor *-Genome-*

Instruction Manual

(Code No.NPK-101,102)

Distributor

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Caution

All the reagents included in this kit are for experimental use and are never to be used for diagnostical or clinical purposes. When using this kit, make sure to follow common laboratory safety procedures carefully.

Hoffman-La Roche Ltd. retains a patent on the PCR method and special permission may be required from them depending on the stated goals of the experiment undertaken.

1. Introduction

MagExtractor *-Genome-* contains reagents for genomic DNA extraction utilizing the properties of DNA absorption[1) 2)] in silica in the presence of chaotropic agents. Using this kit with the MFX-2100 automated nucleic acid extractor enables the simple automated extraction of highly pure genomic DNA from in vivo samples such as whole blood and cultured cells. This kit can also be used for manual extractions.

Capabilities and features

- Because ethanol sedimentation or centrifugal separation is unnecessary, extraction is possible in a short time.
- Does not use harmful solvents such as phenol or chlorophorm.
- DNA can be extracted directly from whole blood rendering unnecessary complex white and lymphatic corpuscle separation.
- Extracted DNA can be collected in sterilized water enabling its immediate use for a PCR or other analytical means.

2. Cautions prior to use

MagExtractor *-Genome-* enables extraction mainly from the following samples:

Sample	Yield Point	Purity (A_{260}/A_{280} ratio)	Application
Blood	2μg/100μl whole blood	1.8±0.1	PCR
Culture cells	3μg 5×10^5cell (HeLa)		
Tissue	5μg/5mg tissue (swine testes)		
Mouse tail	3μg/2mm tail		

- For tissue and mouse tail, pretreatment is required (homogenization and dissolution). See P. 3 for details.
- The yield varies depending on the type of sample and its condition. Please consider the values stated in this document as merely a guideline.
- In addition, with proper pretreatment, studies have shown that extraction from paraffin embedded tissue section, yeast, lactic acid bacilli, etc. may be possible. (For more information, please contact our Technical Line.)
- Please use this kit at room temperature (20 to 30°C). Low or high temperatures may hinder this kit's overall performance.

3. Items included in this kit

The following reagents are included in this kit.

NPK-101 (100 batches)

100ml ----- Lysis & Binding Solution (with protein denaturant) ---------- 4°C
200ml ----- Washing Solution (with protein denaturant) ---------- 4°C
6ml ----- Magnetic Beads ---------- 4°C

NPK-102 (500 batches)

500ml ----- Lysis & Binding Solution (with protein denaturant) ---------- 4°C
500ml× 2 ----- Washing Solution (with protein denaturant) ---------- 4°C
25ml ----- Magnetic Beads ---------- 4°C

・Store all of the above at 4°C and return them to room temperature immediately prior to use. For short storage periods (under 1 month), storing at room temperature (25°C or less) is possible.
・The solvent, adsorption solution and cleansing solution I have a high concentration of protein denaturant. Handle with care and wear gloves along with other protective gear and implement safety precautions. In the event of accidental contact with the skin, rinse off well. If one of these finds its way into the eyes, rinse them immediately with water and see a doctor.
・If using our MFX-2100 automated nucleic acid extractor, dispense the required amount in the designated tubes and set in the predetermined location. For details, refer to the MFX-2100 instruction manual.

4. Protocol

[1] Required items not included in this kit

(1) Reagents

・Sterilized water (commercially available purified water or autoclave sterilized mili-Q water)
・Ethanol (high-grade 99.5% proof for automated extraction using the MFX-2100 and 70% ethanol for manual extraction)

(2) Devices and equipment

・Micropipette
・Micropipette chips
・Homogenizer for micro-tubes
・Tabletop centrifuge (with approx. 3,000 to 10,000 rpm capability)

When using the MFX-2100:

・Extraction specialized tubes (Code No.: MFX-301 or MFX-302)
・Chip with special filter attached (Code No.: MFX-402A)
・Reagent setting tubes (50ml, 15ml, 2ml) *1

When using the manual method

・1.5ml micro-tube
・1.5ml micro-tube magnetic stand (commercially available)
・Tube mixer (MT-360 made by Tomy Seiko Co., Ltd)

*1. The following items are recommended for use as the reagent setting tube: 50ml tube: Blue Max 2170 (Becton Dickinson), 15ml tube: Blue Max, Jr. (Becton Dickinson), 2ml micro-tube: 2220, 2200 (Ina･Optika), 72.693, 72.694 (Assist)

[2] Pre-treatment method for samples

(1) Whole blood

Dispense uniformly mixed 100μl of blood in the sample tubes*1.

・Apart from fresh blood collected immediately before examination, blood treated for anticoagulation with EDTA or heparin or thawed out blood from frozen storage may be used.*2

・If the amount of whole blood is less than 100μl, add Lysis & Binding Solution until a volume of 100μl is reached.

(2) Culture cells

Dispense the culture cells (1×10^5 to 1×10^6 cells equivalent) collected in PHS (phosphate-buffered saline) in assist tubes (No. 72.692) or 1.5ml microtubes and, after centrifuging (6,000 rpm for 5 minutes), remove the supernatant.

・For a cell density of 1×10^5 to 1×10^6 cells/100μl, dispense 100μl from the whole into tubes and set them onto the tube rack.

*1. For the sample tubes, apart from using tubes especially made for the MFX-2100 (Code No.: MFX-301), assist tubes (No. 72.692) or 1.5ml microtubes may also be used.

*2 If storing blood for long periods, make sure to check for coagulation. Clotting may cause chip clogging.

*3 Depending on the type and condition of the tissue, conducting the pretreatment described in ② may result in an efficient extraction.

(3) Tissue, Mouse tail

Dissolve the sample using any of the methods described below. For substantial tissue samples such as liver tissue, any of the following methods will result in the acquisition of genomic DNA of roughly equivalent extraction efficiency and purity.*3

① Dissolving the sample using Lysis & Binding Solution

Tissue section (10mg or less) or mouse tail (2 to 5mm)

↓

←850μl Lysis & Binding Solution
Homogenization (perform well using a homogenizer for microtubes)
Centrifuge (10,000 rpm for 5 minutes)

Supernatant (approx. 850μl)

・Dispense the entire amount of supernatant in the sample tubes.

・Should the dispensed amount be less than 850μl, add Lysis & Binding Solution until 850μl is reached.

② Dissolving samples using proteinase K digestion

Tissue section (10mg or less) or mouse tail (2 to 5mm)

↓

←90μl Proteinase K buffer (100mM NaCl, 10mM Tris-HCl(pH8.0), 25mM EDTA)
←5μl 10mg/ml Proteinase K(30U/mg)
←5μl 10% SDS
Apply 55°C of heat for 6 to 18 hours (mix 2 to 3 times during this operation to ensure sufficient digestion)
Centrifuge (10,000 rpm for 5 minutes)

Supernatant (approx. 100μl)

・Dispense the entire amount of supernatant in the sample tubes.

・Should the dispensed amount be less than 100μl, add Lysis & Binding Solution until 100μl is reached.

[3] Extraction flow

Extraction flow using the MagExtractor -*Genome*- is described below:

Sample
↓
←Lysis & Binding Solution ---------- [dissolution of the sample]
←Magnetic Beads ---------- [Genomic DNA absorption]
B/F separation
←Washing Solution ---------- [excluding non-specific adsorbate]
B/F separation
←70% Ethanol ---------- [excluding protein denaturant]
B/F separation
←eluate (sterilized water) ---------- [DNA elution from magnetic beads]
B/F separation
↓
Supernatant collection (100μl)

[4] Genomic DNA extraction using the MFX-2100

Before using the MFX-2100, make sure to read its operation manual thoroughly.

(1) Selecting protocols

3 Genomic DNA extraction protocols for the MFX-2100 have been prepared. Select either of the following according to need*1 and enter the protocol number while observing the liquid crystal display.

Protocol	Entry No.	Displayed description	Sample (volume)
Blood	11	Genome: Blood	liquids (100μl) such as blood, Proteinase K digested, etc.
Cultured cell	12	Genome: Cell	Cell pellet such cultured cell, etc.
Tissue	13	Genome: Tissue	Lysate (850μl) such as tissues

- Only one protocol can be selected for one experiment.
- See P. 3 for the pretreatment methods of each sample.
- For cultured cells collected in PBS with a cell density of 1×10^5 to 1×10^6 cells/100μl, use 100μl of the whole under the blood protocol (entry number: 11).
- For tissue or mouse tail pretreated as per ① (dissolved with Lysis & Binding Solution), select the tissue protocol (entry number: 13). If pretreated as per ② (dissolved with proteinase K), select the blood protocol (entry number:11)
- Roughly 11 minutes per sample is necessary for extraction. Extraction time varies as per the sample count.

*1 The 3 types of genomic DNA extraction protocols optimize the sample dissolution process parameter conditions depending on each respective sample amount (liquid volume) and pretreatment method. As such, if extracting from identical samples, select protocols for each one that are best suited with respect to volume and pretreatment method.

(2) Heating block and cooling block temperature setting

Set the cooling blocks at the temperatures stated below:

	Heating block	Cooling block
Temperature setting	OFF	10°C

- The heater block is not used. Please turn its power supply OFF.

(3) Setting chips with special attached filter

Set chips with special attached filter (Code No.: MFX-402A) to the chip rack. Refer to the following table for quantities.

- Make sure to use chips with special attached filter (Code No.: MFX-402A).
- The chips are electron irradiation sterilized. Wear gloves when setting the required number onto the chip rack.
- You may set more chips than the quantities prescribed below.
- For chip setting positions, consult the MFX-2100 instruction manual.

		Chip count					Chip count		
Protocol No.		11	12	13	Protocol No.		11	12	13
Sample count	1	7	7	6	Sample count	13	40	40	39
	2	9	9	8		14	42	42	41
	3	11	11	10		15	44	44	43
	4	13	13	12		16	46	46	45
	5	18	18	17		17	51	51	50
	6	20	20	19		18	53	53	52
	7	22	22	21		19	55	55	54
	8	24	24	23		20	57	57	56
	9	29	29	28		21	62	62	61
	10	31	31	30		22	64	64	63
	11	33	33	32		23	66	66	65
	12	35	35	34		24	68	68	67

(4) Setting the special tubes

Set the special tubes (Code No.: MFX-301 or MFX-302) in A to E of the extraction rack, the heating and cooling blocks for the number of samples.

- Do not use items other than specially made tubes for the extraction rack. Doing so may cause problems.
- Screw cap type 1.5ml tubes *1 can be set in the collection block.
- Refer to the MFX-2100 operation manual for more details on setting method.

*1 Assist tubes (No. 72.692), etc.

(5) Setting reagents

Transfer the reagent into the tubes of designated sizes and set them onto the reagent rack in place.

- The reagent amount needed varies depending on the sample count. Set the reagents as per the table on the following page.
- After having rigorously agitated the magnetic beads, verify that they have suspended uniformly and dispense them into 2ml tubes. Also, start the device immediately after having set them (within 10 minutes). Not doing so causes fluctuating yields or operational malfunctions.
- Use a pipette for dispensing the magnetic beads. For other reagents, you may use the gradients on the reagent tubes as a guide.
- The remaining reagent after extraction can be reused. If storing for an extended period, take note that the composition of the solution may change depending on the level of dryness.
- Sterilized water and ethanol (high-grade 99.5% proof) are not included in this kit.
- If selecting protocol #13, setting solvent/adsorption solution is unnecessary.

Reagent rack			Protocols		
Set position	Reagent name	Tube	11	12	13
1	Sterilized water	50ml	Req.	Req.	Req.
2	Washing Solution	50ml	Req.	Req.	Req.
4	Lysis & Binding Solution	50ml	Req.	Req.	Not req.
5	Ethanol	50ml	Req.	Req.	Req.
7	Magnetic Beads	2ml	Req.	Req.	Req.
9	Sterilized water	15ml	Req.	Req.	Req.

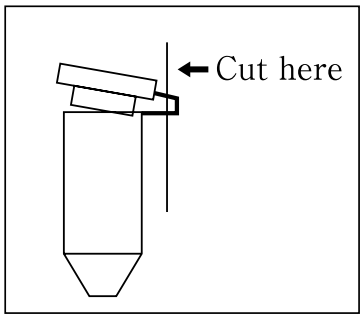
Dispersal amount for each reagent

Reagent		Sterilized water	Washing Solution	Lysis & Binding Solution	Ethanol	Magnetic Beads	Sterilized water
Tube size		50ml	50ml	50ml	50ml	2ml	15ml
Set position		1	2	4	5	7	9
		Amount of reagent dispensed to reagent tubes (ml)					
Quantity of samples	1	5 (20)	5 (20)	5 (20)	5 (20)	0.5 (1.5)	2 (5)
	2						
	3		10 (20)		10 (20)		
	4						
	5		15 (20)	10 (20)		0.8 (1.5)	
	6	10 (20)			15 (20)		3 (5)
	7						
	8		20 (20)				
	9						
	10				20 (20)		
	11		25 (35)	15 (20)			
	12						
	13					1 (1.5)	
	14		30 (35)		25 (35)		
	15						
	16	15 (20)	35 (35)				4 (5)
	17			20 (20)			
	18				30 (35)	1.3 (1.5)	
	19		40 (45)				
	20						
	21						
	22		45 (45)		35 (45)		
	23			25 (35)		1.5 (1.5)	
	24						

- A reference guideline for the dispensing amount for each reagent as per the sample count appears in the above table. These include a surplus, therefore, dispense the reagents in the reagent tubes as per these values.
- The values in parentheses represent the maximum permissible amount standard for when dispensing into the reagent tubes. Dispensing amounts exceeding these values may cause soiling of the nozzle, spills or other problems.

(6) Pre-treatment of samples and settings

Prepare the samples as per the pretreatment methods described on P. 3 and set them onto the extraction rack.



- If using screw cap type 1.5ml sample tubes, set them in their positions with the caps removed.
- When using a normal 1.5ml tube, cut the cap off carefully with scissors and set. Make sure to exercise caution when cutting.
- Set in order as per the holes numbered in front from 1 to 24.

(7) Initiate extraction

Start the extraction as per the following criteria.

- Verify whether the samples, reagent tubes, specialized tubes (extraction rack and collection block) and specialized chips are set as per the prescribed instructions.
- Verify the set position of each rack and whether the stage is completely housed in the back. Close the front cover.
- Enter the protocol number and the number of samples in the liquid crystal display screen.
- Verify whether "press start key" is displayed on the screen and press "START".
- Extraction initiates and the liquid crystal display screen shows "in operation".

(8) Collect samples

- When the extraction is complete, collect the extracted DNA into the tubes set onto the collection block. Also, "Press the start key" will reappear in the liquid crystal display.
- After verifying whether the device has completely stopped, remove the tubes from the collection block and place on ice or store in a low temperature chamber (4 to 10°C) until ready for use.
- For the elution of DNA from the magnetic beads, use 100μl of solvent (sterilized water). The collected volume will be around 100μl.

[5] Genomic DNA extraction using the manual method

This kit may also be used for manual DNA extraction*1. Perform extraction as per the following order with respect to the type of sample.

- Preparation order varies as per the sample type. For details, see "Sample pretreatment methods" on P. 3.
- For magnetic bead separation, the use of a commercially available magnetic stand made for 1.5ml microtubes is recommended. However, using a simple tabletop centrifuge at 3,000 rpm for 5 seconds can also enable adequate separation.
- Also needed with this kit is 70% ethanol and sterilized water. Prepare 70% ethanol in advance by mixing high-grade ethanol with sterilized water at a ratio of 7:3 (1.8ml is needed per sample).
- Perform all extraction operations at room temperature.

*1 By making the entire amounts of reagent and sample proportional to each other and increasing or decreasing the corresponding amounts, the extraction scale may be changed.

(1) Whole blood

① Dispense 100μl of blood into 1.5ml microtubes

② After adding 750μl of Lysis & Binding Solution and 40μl of Magnetic Beads, mix well for 10 minutes using a tube mixer.
- Use only after having mixed well the magnetic beads in advance.
- Adjust the agitation speed to ensure sufficient mixing of the sample and magnetic beads.

③ Set the tubes onto the magnetic stand and, by leaving for 30 seconds or so, the magnetic beads will be drawn together. Then, remove the supernatant.
- If there are magnetic beads adhering to the inside of the tube caps, tumble mix the magnetic stand 3 times with the tubes still set in it to collect them completely together.
- With the tubes set onto the magnetic stand, use a micro-pipette to remove the most supernatant possible.
- Remove the most supernatant possible all the while being cautious not to suck in any magnetic beads.

④ Add 900μl of Washing Solution in the tubes and rigorously agitate for 5 seconds using a vortex mixer.
- Perform this operation until the magnetic beads are uniformly scattered.

⑤ Set the tubes onto the magnetic stand and leave for 30 seconds to let the magnetic beads get drawn together. Then, remove the supernatant.
- Perform exactly as ③.

⑥ Repeat steps, ④ and ⑤.

⑦ After having added 900μl of 70% ethanol, use a vortex mixer and stir well for 5 seconds.
- Perform this operation until the magnetic beads are uniformly scattered.

⑧ Set the tubes onto the magnetic stand and, by leaving for 30 seconds or so, the magnetic beads will be drawn together. Then, remove the supernatant.
- Perform exactly as ③.

⑨ Repeat steps, ⑦ and ⑧.

⑩ After having added 100μl of sterilized water, use a tube mixer and stir for 10 minutes. This will elute the genomic DNA.
- Insert sterilized water in the bottom of the tubes all the while being extra cautious not to have any come into contact with the tube walls.
- As with ②, adjust the stirring speed to ensure sufficient mixing of the sample and magnetic beads. However, adjust in a way so as to prevent the solution from splashing up onto the inside of the tube caps.

⑪ Set the tubes onto the magnetic stand and leave unattended for 30 seconds so as to let the magnetic beads get drawn together. Then, collect the genomic DNA containing supernatant into a new 1.5ml microtube.

[Extraction flow]

100μl Blood

←750μl Lysis & Binding Solution
←40μl Magnetic Beads (tumble stirred uniformly mixed)
Stir 10 minutes
B/F separation
←900μl Washing Solution
Stir 5 seconds
B/F separation
(repeat twice)
←900μl 70% Ethanol
Stir 5 seconds
B/F separation
(repeat twice)
←100μl Sterilized water
Stir 10 minutes
B/F separation

Supernatant

(2) Cultured cells

① After having dispensed cultured cells (1×10^5 to 1×10^6 cells equivalent) collected in PBS (phosphate-buffered saline) into 1.5ml microtubes, centrifuge (6,000 rpm for 5 minutes) and remove the supernatant.

② After adding 850μl of Lysis & Binding Solution and 40μl of Magnetic Beads, mix well for 10 minutes using a tube mixer.
- Use only after having mixed well the magnetic beads in advance.
- Adjust the agitation speed to ensure sufficient mixing of the sample and magnetic beads.

③ Perform procedures [5] (1) ③ to ⑪ (P.8) for whole blood and, lastly, collect the genomic DNA containing supernatant into a new 1.5ml microtube.

[Extraction flow]

PBS collected cultured cells (1×10^5 to 1×10^6 cells)

Centrifuge (6,000 rpm for 5 minutes)
Supernatant removal (cell collection)
←850μl Lysis & Binding Solution
←40μl Magnetic Beads (tumble stirred uniformly mixed)
Stir 10 minutes
B/F separation
←900μl Washing Solution
Stir 5 seconds
B/F separation
(repeat twice)
←900μl 70% Ethanol
Stir 5 seconds
B/F separation
(repeat twice)
←100μl Sterilized water
Stir 10 minutes
B/F separation

Supernatant

(3) Tissue, mouse tail (sample dissolution method using Lysis & Binding Solution)

① Insert tissue section (10mg or less) or mouse tail (2 to 5mm) into the 1.5ml microtubes and, after having added 850μl of Lysis & Binding Solution, use a microtube homogenizer and proceed with ample homogenization.

② After centrifuging (10,000 rpm for 5 minutes), insert the supernatant into a new 1.5ml microtube.

③ Add to this 40μl of Magnetic Beads and mix well for 10 minutes using a tube mixer.

- Use only after having mixed well the magnetic beads in advance.
- Adjust the agitation speed to ensure sufficient mixing of the sample and magnetic beads.

④ ① Perform procedures [5] (1) ③ to ⑪ (P.8) for whole blood and, lastly, collect the genomic DNA containing supernatant into a new 1.5ml microtube.

[Extraction flow]

Tissue section (10mg or less) or mouse tail (2 to 5mm)
- ←850μl Lysis & Binding Solution
- Homogenize sufficiently
- Centrifuge (10,000 rpm for 5 minutes)
- Dispense the supernatant into a 1.5ml tube
- ←40μl Magnetic Beads (tumble stirred uniformly mixed)
- Stir 10 minutes
- B/F separation
- ←900μl Washing Solution
- Stir 5 seconds
- B/F separation

(repeat twice)
- ←900μl 70% Ethanol
- Stir 5 seconds
- B/F separation

(repeat twice)
- ←100μl Sterilized water
- Stir 10 minutes
- B/F separation

Supernatant

(4) Tissue, mouse tail (samples dissolved using proteinase K)

① Insert tissue section (10mg or less) or mouse tail (2 to 5mm) into 1.5ml microtubes and add, in order, 90μl of proteinase K buffer (100mM NaCl, 10mM Tris-HCI (pH 8.0), 25mM EDTA), 5μl of 10mg/ml proteinase K (30U/mg) and 5μl of 10% SDS and apply 55°C of heat for 6 to 18 hours.

- Mix 2 or 3 times midway to ensure sufficient digestion.

② After centrifuging (10,000 rpm for 5 minutes), insert the supernatant into a new 1.5ml microtube.

③ After adding 750μl of Lysis & Binding Solution and 40μl of Magnetic Beads, mix well for 10 minutes using a tube mixer.

- Use only after having mixed well the magnetic beads in advance.
- Adjust the agitation speed to ensure sufficient mixing of the sample and magnetic beads.

④ Perform procedures [5] (1) ③ to ⑪ (P.8) for whole blood and, lastly, collect the genomic DNA containing supernatant into a new 1.5ml microtube.

[Extraction flow]

Tissue section (10mg or less) or mouse tail (2 to 5mm)

←90μl Proteinase K buffer(100mM NaCl, 10mM Tris-HCl(pH8.0), 25mM EDTA)
←5μl 10mg/ml Proteinase K(30U/mg)
←5μl 10% SDS
55°C, Apply heat for 6 to 18 hours
Centrifuge (10,000 rpm for 5 minutes)
Dispense the supernatant into a 1.5ml tube
←750μl Lysis & Binding Solution
←40μl Magnetic Beads (tumble stirred uniformly mixed)
Stir 10 minutes
B/F separation
←900μl Washing Solution
Stir 5 seconds
B/F separation
(repeat twice)
←900μl 70% Ethanol
Stir 5 seconds
B/F separation
(repeat twice)
←100μl Sterilized water
Stir 10 minutes
B/F separation

Supernatant

[6] Post-extraction analysis

(1) DNA quantitative determination

The amount of DNA present in the collected solution can be determined by way of light absorption rate measurement with 260nm wavelengths.

- When measuring light absorption, make sure to centrifuge the collected solution (10,000 rpm for 1 minute) and use the resulting supernatant.
- When calculating the A_{260nm}/A_{280nm} ratio and the DNA concentration, make sure to compensate using A_{320nm} value as a background.

(2) Electrophoresis

Mix into the collected solution electrophoresis loading dye that was either bought on the market or prepared in-house. Then, apply agarose gel.

- When applying, make the amount of loading dye 1.5 to 2 times the amount normally used. There may be instances where the sample for migration does not settle inside the well rendering normal application impossible.

(3) PCR

By performing a PCR using part of the collected solution as a sample template, it is possible to use the DNA contained in the collected solution as a target.

- A small amount of magnetic beads remaining in the collected solution will not hinder the PCR.
- The collected solution contains 10% of ethanol. If using for a PCR, this may inhibit the reaction. Therefore, do not exceed 1/5 of the reaction solution amount (i.e.: for a 50μl reaction solution, use 10μl).

5. Trouble shooting

Refer to the following countermeasures should any troubles occur.

[1] Low yield and no DNA extracted

Cause	Remedy
Excessive sample	Even if using more than the prescribed sample amount, the yield does not increase but rather drops. Use the correct sample amount.
Insufficient pulverization and dissolution	(When using tissue or mouse tail) This may mean that there was insufficient sample pretreatment (P. 3). For the pretreatment method described in (1) (dissolution via Lysis & Binding Solution), take ample time (at least 5 minutes) and homogenize enough so that the coagulation in the tissue disappear. Also, part of formalin hardened tissue samples pretreated with the method in (1) may not dissolve. Try instead method (2) (dissolution by proteinase K).
	(When using gram-positive bacteria) Samples such as gram-positive bacteria may not dissolve with the Lysis & Binding Solution included with this kit. For such cases, a special dissolving method is necessary. For example, in the case of yeast, it is necessary to dissolve the cell walls in advance using dissolving enzymes such as zymolyase.
Insufficient reagent amount	When performing an automated extraction using the MFX-2100, check the entire reagent amount remaining to see whether the amount used was insufficient. If there is almost none remaining (200μl or less), this means there was an insufficient amount.

・The yield changes with respect to the sample type and condition.

[2] Unsuccessful PCR

Cause	Remedy
Inhibition due to ethanol contamination in the collected solution	Using more than 1/5 the reaction solution may hinder the reaction. For such a case, decrease the solution amount. Also, applying 75°C of heat for 5 minutes may bring about better results.
Target size too long	The average strand length for extracted DNA is 40kb (for whole blood DNA), a length suitable for use as a common PCR template. However, for a target size exceeding 5kb, the PCR enzyme performance may dwindle causing an unfavorable amplification. In such cases, using long distance PCR enzymes such as the KOD Dash DNA Polymerase (Code No.: LDP-101) is recommended.

・For other PCR troubleshooting tips, refer to our collection of PCR enzyme working examples as well as guides pertaining to the PCR.

[3] The beads don't completely scatter when cleansing and they clog up the chips
(when performing automated extraction using the MFX-2100)

Cause	Remedy
Surplus sample	If there is excessive sample amount, the beads may be coagulating. Also, even if using more than the prescribed amount, the yield will not increase but rather drop. Decrease the sample amount and reattempt.
Insufficient reagent	Check the entire reagent amount to see whether there was an insufficient reagent amount. If there is almost none left (200μl or less), this means that the amount was insufficient. Low cleansing solution may result in insufficient stirring which may cause the beads not to scatter.

[4] Unfavorable dispensing of the beads
(when performing an automated extraction using the MFX-2100)

Cause	Remedy
Solidification due to the settling of the magnetic beads	After having suspended until having reached uniformity using a vortex, perform extraction again. Before starting the extraction, use a vortex until the solution is uniformly suspended or move the magnetic beads to a 2ml tube immediately before starting (within 10 minutes).
Condensation and crystallization of the magnetic beads suspended solution elements due to evaporation	To avoid this problem, throw away the beads remaining in the 2ml tubes. Also, if storing or leaving unused for a short time, firmly close the lids of the bottles or tubes.

・For other operational troubleshooting tips, refer to the MFX-2100 instruction manual and use it in conjunction with this document.

6. References

1) Vogelstein, B., and Gillespie, D. (1979) Preparative and analytical purification of DNA from agarose. *Proc. Natl. Acad. Sci.* **76**: 615-619.
2) Boom, R., Sol, C.J.A., Salimans, M.M.M., Jansen, C.L., Wertheim-van Dillen, P.M.E., and van der Noordaa, J. (1990) Rapid and simple method for purification of nucleic acids. *J. Clin. Microbiol.* **28**: 495-503.

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