FUJIFILM

Wako

Code No. 292-81101（For 50 tests）

残留 DNA 提取试剂盒（碘化钠法）

本产品遵照中国药典记载的碘化钠法，属于宿主细胞残余 DNA 抽提试剂盒。请用于疫苗和治疗用生物制品中含有的宿主细胞残余 DNA 的提取。
本试剂盒可以提取样品中含有的极微量的 DNA，回收率较高。DNA 的提取操作时间为 60-90min。提取的 DNA 可以通过 qPCR 法进行定量。

本试剂盒提供的标准步骤适用于从原料样品（如细胞裂解物，或含有高浓度蛋白质或脂质的溶液）中提取 DNA。同时我们也提供了简化步骤，用于从提纯样品（如纯化蛋白质）中提取 DNA，两种操作方法的不同点见下表。

	样品	样品 预处理步骤	洗涤步骤	试剂盒中 “洗净液 A，CP”
标准步骤	原料样品 （细胞裂解物、含高浓度蛋白或脂质的溶液）	需要	2 次	使用
简化步骤	提纯样品 （纯化蛋白血清）	不需要	1 次	不使用

【特点】

・遵照中国药典记载的碘化钠法
・高回收率
・单管提取 DNA（无需更换管）

【通过碘化钠法提取 DNA 的原理】

通过碘化钠和 N- 月桂酰肌氨酸钠，将样品中的蛋白质及脂质等溶解。再添加异丙醇，使 DNA 与糖原共同沉淀。此时离液离子的碘化钠及 N- 月桂酰肌氨酸钠阻碍样品中的蛋白质及脂质等成分沉淀，DNA 及糖原发生特异性沉淀。

【试剂盒构成】

产品编号	组分	规格
299-81111	碘化钠溶液 , CP	26mL×1
296-81121	N- 月桂酰肌氨酸钠溶液 , CP	1.2mL×1
293-81131	洗净液 A, CP	42mL×1
290-81141	洗净液 B, CP	40mL×2
297-81151	糖原溶液 , CP	0.1mL×1

【存储】

2-10℃避光保存。

【50 次试验所需的附加材料】

< 试剂 >
1）异丙醇　20mL
2）无菌蒸馏水　6mL

< 仪器 >
1）微型离心机
2）涡旋混合器
3）50×2mL 离心管
4）微量移液器

5）微量移液器吸头
6）加热器

【使用前】

●“洗净液 A，CP”根据批次不同，溶液颜色有可能存在差异（无色～浅黄色），产品性能没有问题，属正常现象。
●在“碘化钠溶液，CP”或“N-月桂酰肌氨酸钠溶液，CP”产生沉淀物时，请在 50℃条件下加热至沉淀物溶解。
●请使用未受 DNA或 DNase污染的试剂及器具。
●如果提取的 DNA中残留有碘化钠，将导致无法通过吸光度准确测量 DNA浓度。请通过 qPCR法将 DNA定量。

【标准步骤】

< 试剂的调制 >

1．溶液 I
在 26mL 的“碘化钠溶液，CP”容器中添加 65μL 的“糖原溶液，CP”，利用涡旋混合器充分混合均匀。
备注：在 2-10℃避光保存条件下，溶液 I 稳定状态可保存约 5 个月。

2．溶液Ⅱ
在 40mL“洗净液 B，CP”容器中添加 2μL“糖原溶液，CP”。立即使用涡旋混合器充分混合均匀。
备注：
・此时，即使溶液 II 中产生白色沉淀物也不会影响 DNA 的提取质量。
・在 2-10℃避光保存条件下，溶液 II 稳定状态可至少保存约 1 周。制备少量时，只需调制所需量的“糖原溶液，CP”和“洗净液 B，CP”即可。例如，在 20mL“洗净液 B，CP”无菌容器中添加 1μL“糖原溶液，CP”。

< 样品制备 >

■样品溶液中蛋白质浓度小于 2mg/mL 时
1．将下列试剂加入样品溶液至最终浓度。

试剂	最终浓度
十二烷基硫酸钠 (SDS)	0.10%
二硫苏糖醇 (DTT)	50mmol/L

2．在加热器中加热（55℃）30min。

■样品溶液中蛋白质浓度大于 2mg/mL 时
1．将下列试剂加入样品溶液至最终浓度。

试剂	最终浓度
十二烷基硫酸钠 (SDS)	0.10%
二硫苏糖醇 (DTT)	50mmol/L
氯化钠 (NaCl)	150mmol/L
乙二胺四乙酸（EDTA）	1mmol/L

2．将蛋白酶 K 按照样品溶液中每 1mg 蛋白质 20μg 的比例加入样品溶液。
3．采用 Tris-HCl 将 pH 调至约 7.5。
4．在加热器中加热（55℃）至少 1h。

<DNA 的提取 >

1．吸取 400-500μL 样品溶液加入 2mL 空离心管。
2．添加 20μL“N- 月桂酰肌氨酸钠溶液，CP”至该离心管，并使用涡旋混合器混合均匀。
3．添加 500μL 溶液 I 至该离心管，并使用涡旋混合器混合均匀。
4．在加热器中将离心管在 40℃条件下加热 15min。
5．从加热器中取出离心管。加入 900μL 异丙醇至离心管，并使用涡旋混合器混合均匀。
6．离心管在室温下静置 15min。

7．将离心管进行离心（10,000×g，15min，室温）。出现白色片状沉淀。
8．用移液器移除或小心倾倒管中上清液，直至管中液体容积小于约 100μL。
备注：
・请注意不要去除管中片状沉淀。
・若有片状沉淀漂浮，再次将离心管进行离心（10,000×g，15min，室温）。
・若溶液残留在管壁，将离心管倒置于滤纸以移除残留液体。
9．添加 800μL“洗净液 A，CP”至该离心管，并进行颠倒混合以确保片状沉淀从管壁脱落。
10．将离心管进行离心（10,000×g, 5min，室温）
11．用移液器移除或小心倾倒管中上清液，直至管中液体容积小于约 100μL。
备注：
・请注意不要去除管中片状沉淀。
・若有片状沉淀漂浮，再次将离心管进行离心（10,000×g，5min，室温）。
・若溶液残留在管壁，将离心管倒置于滤纸以移除残留液体。
12．添加 1.5mL 溶液 II 至该离心管，再颠倒混合。
13．将离心管进行离心（10,000×g，5min，室温）。
14．用移液器或小心倾倒的方式尽可能多地移除管中的上清液。
备注：
・请注意不要去除管中片状沉淀。
・若有片状沉淀漂浮，再次将离心管进行离心（10,000×g，5min，室温）。
・若溶液残留在管壁，将离心管倒置于滤纸以移除残留液体。
15．将片状沉淀置于加热器、真空干燥器中加热（60℃）使其干燥，或使其自然干燥。
备注：
・若过分干燥的话，将导致 DNA 重新溶解困难。当片状沉淀呈湿润的状态时，停止干燥。
・片状沉淀中含有 DNA 和糖原。
・由于溶液 II 包含可以抑制 qPCR 的乙醇，管中残留液体过多会降低 qPCR 效率。
16．将片状沉淀在无菌蒸馏水或缓冲液中重新溶解，用于 qPCR。

【简化步骤】

本方法用于杂质含量相对较低的纯化蛋白和血清样品的 DNA 提取。
本方法中样品省去了前处理部分，洗涤步骤只有一步，简化了操作过程，减少了操作时间。

＜试剂的配制＞

1．溶液Ⅰ（简化版）
在 26mL 的“碘化钠溶液，CP”容器中添加 6mL“无菌蒸馏水”、1mL“N- 月桂酰肌氨酸钠溶液，CP”、65μL“糖原溶液，CP”共计三种。利用涡旋混合器充分混合均匀。
备注：在冷藏保存条件下，溶液Ⅰ（简化版）稳定状态可保存约 5 个月。

2．溶液Ⅱ
在 40 mL“洗净液 B, CP”容器中添加 2μL“糖原溶液，CP”。立即使用涡旋混合器充分混合均匀。
备注：
・此时，即使产生白色沉淀物也不会影响 DNA 的提取效率。请直接使用。
・在冷藏保存条件下，溶液 II 稳定状态可保存约 1 周。在少量使用时，只需调制所需量即可。例如：20mL“洗净液 B, CP”中添加 1μL“糖原溶液，CP”。

＜DNA 的提取＞

1．吸取 100μL 样品加入 1.5mL 或 2mL 离心管。

2．再添加 300μL 配制好的溶液Ⅰ（简化版）至该离心管，并使用涡旋混合器混合均匀。
3．将离心管在 60℃条件下加热 15min。
4．取出离心管，加入 400μL 异丙醇至离心管，并使用涡旋混合器混合均匀。
5．离心管在室温下静置 15min。
6．将离心管进行离心（10,000g，15min，室温）。出现白色片状沉淀。
7．用移液器移除或小心倾倒管中上清液，直至管中液体容积小于约 100μL。
备注：
・请注意不要去除片状沉淀
・若有片状沉淀漂浮，再次将离心管进行离心（10,000g，15min，室温）。
・若溶液残留在管壁，将离心管倒置于滤纸以移除残留液体。
8．在离心管中添加 1mL 溶液 II，充分颠倒混合，使白色片状沉淀从离心管上剥离。
9．将离心管进行离心（10,000g，5min，室温）。
10．用移液器或小心倾倒的方式尽可能多地移除管中的上清液。
备注：
・请注意不要去除片状沉淀。
・若有片状沉淀漂浮，再次将离心管进行离心（10,000g，5min，室温）。
・若溶液残留在管壁，将离心管倒置于滤纸以移除残留液体。
11．将片状沉淀置于加热器、真空干燥器中加热（60℃）使其干燥，或使其自然干燥。
备注：
・若过分干燥的话，将导致 DNA 重新溶解困难。当片状沉淀呈湿润的状态时，停止干燥。
・片状沉淀中含有 DNA 和糖原。
・由于溶液 II 包含可以抑制 qPCR 的乙醇，管中残留液体过多会降低 qPCR 效率。
12．将片状沉淀在无菌蒸馏水或缓冲液中重新溶解，用于 qPCR。

【FAQ】

Q. 利用吸光度测量提取的 DNA 浓度时，在 230nm 波长处出现波峰。
A. 因为和 DNA 共沉淀的碘化钠在 230nm 处有吸收峰，虽然残留有碘化钠，但对 qPCR 无影响。

Q. DNA 的回收率低
A. 在 DNA 提取操作步骤 7. 和 10. 中极有可能一部分片状沉淀被除去。请使用移液器谨慎去除上清液。

<Reference>
Ishizawa, M., Kobayashi, Y., Miyamura, T. and Matuura, S. (1991) Simple Procedure of DNA isolation from human serum, *Nucleic Acids Res.*, **19**, 5792.

FUJIFILM Wako Pure Chemical Corporation
1-2, Doshomachi 3-Chome, Chuo-Ku, Osaka 540-8605, Japan
Telephone ：+81-6-6203-3741
Facsimile ：+81-6-6201-5964

FUJIFILM Wako Chemicals (Hong Kong) Limited
Room 1111, 11/F, International Trade Centre, 11-19 Sha Tsui Road, Tsuen Wan, N.T., Hong Kong
Telephone ：+ 852-2799-9019
wkhk.info@fujifilm.com

富士胶片和光（广州）贸易有限公司
广州市越秀区先烈中路 69 号东山广场 3003 室（510095）
电话：+86 20 8732 6381（广州）
电话：+86 21 6288 4751（上海）
电话：+86 10 6413 6388（北京）
wkgz.info@fujifilm.com

https://labchem-wako.fujifilm.com

Code No. 292-81101 (For 50 tests)

DNA Extractor™ Kit for Residual DNA, CP Method (Sodium Iodide Method)

This product is in compliance with sodium Iodide method and designed for use in extracting residual host cell DNA in biological samples such as vaccines and biopharmaceuticals.
It offers high recovery of DNA even from samples containing only a very small amount of DNA. The entire extraction step is completed with a single tube for 60-90 minutes. The extracted DNA can be quantified by qPCR.

The standard protocol provided in this kit is suitable for extracting DNA from raw material samples (eg. cell lysates, or solutions containing high concentrations of proteins or lipids). At the same time, we also provide simplified steps for extracting DNA from purified samples (eg. purified proteins and serum). The differences between the two operating methods are shown in the table below.

	Sample of interest	Pretreatment step of sample	Number of washing steps	"Washing Solution A, CP" in the kit
Standard Protocol	Crude	Required	2 times	Used
Simplified Protocol	Clean	Not required	1 time	Not used

【Features】

・In compliance with sodium Iodide method
・High quality and recovery
・Completed in a single tube for 60-90 minutes.

【Principle of sodium Iodide method for DNA extraction】

An chaotropic reagent, Sodium Iodide, and an anionic detergent participate in solubilization of proteins and lipids contained in biological samples. After addition of 2-propanol to the mixture, nucleic acids are co-precipitated with glycogen as a carrier, while other components remain soluble in the solution phase.

【Kit contents】

Code No.	Components	Size
299-81111	Sodium Iodide Solution, CP	26 mL × 1
296-81121	Sodium N-Lauryl Sarcosinate Solution, CP	1.2 mL × 1
293-81131	Washing Solution A, CP	42 mL × 1
290-81141	Washing Solution B, CP	40 mL × 2
297-81151	Glycogen Solution, CP	0.1 mL × 1

【Storage】

Store at 2-10℃ in the dark.

【Additional materials required for 50 tests】

<Reagents>
1) 2-Propanol 20 mL
2) Sterile distilled water 6 mL

<Instruments>
1) Microcentrifuge
2) Vortex mixer
3) 50×2 mL centrifuge tube
4) Micropipette
5) Micropipette tips
6) Heating block

【Precautions for Use】

● The color of "Washing Solution A, CP" may be slightly different between product lots. e.g. faint yellow or colorless. It does not affect quality of DNA extraction.
● If precipitate is observed in "Sodium Iodide Solution, CP" or "Sodium N-Lauryl Sarcosinate Solution, CP", warm it or them at 50℃ until the precipitate is no longer visible.
● Use DNA-free, DNase-free and sterilized reagents and Instruments.
● DNA concentration cannot be accurately measured by absorbance if sodium Iodide remains in the extracted DNA. Measure the amount of DNA by qPCR

【Standard Protocol】

<Preparation of Reagents>

1. Solution I
Add 65 μL of "Glycogen Solution, CP" into the bottle of 26 mL of "Sodium Iodide Solution, CP". Mix it with a vortex mixer.
Note : Solution I can be stored at 2-10℃ in the dark for approximately 5 months.

2. Solution II
Add 2 μL of "Glycogen Solution, CP" into the bottle of 40 mL of "Washing Solution B, CP". Mix it with a vortex mixer immediately.
Notes :
・It does not affect quality of DNA extraction if white precipitates appear in Solution II.
・Solution II can be stored at 2-10℃ in the dark for approximately a week at least. If preparing a small volume of Solution II, use required amount of "Glycogen Solution, CP" and "Washing Solution B, CP". For example, add 1 μL of "Glycogen Solution, CP" into 20 mL of "Washing Solution B, CP" in a sterilized tube.

<Preparation of Samples>

■ In case that protein concentration in the sample solution is less than 2 mg/mL.
1. Add the reagents below into the sample solution to the final concentration.

Reagents	Final concentration
Sodium Dodecyl Sulfate	0.10%
Dithiothreitol	50 mmol/L

2. Warm it at 55℃ for 30 minutes in a heating block.

■ In case that protein concentration in the sample solution is more than 2 mg/mL.
1. Add the reagents below into the sample solution to the final concentration.

Reagents	Final concentration
Sodium Dodecyl Sulfate	0.10%
Dithiothreitol	50 mmol/L
Sodium Chloride	150 mmol/L
EDTA	1 mmol/L

2. Add 20 μg of Proteinase K per 1 mg of protein in the sample solution in to the sample solution.
3. Adjust pH to be approximately 7.5 with Tris.
4. Warm it at 55℃ for an hour at least in a heating block.

<DNA Extraction Procedure>

1. Dispense 400-500 μL of sample solution to a 2 mL blank centrifuge tube.
2. Add 20 μL of "Sodium N-Lauryl Sarcosinate Solution, CP" into the tube and mix it with a vortex mixer.
3. Add 500 μL of Solution I into the tube and mix it with a vortex mixer.
4. Warm the tube at 40℃ for 15 minutes in a heating block.
5. Remove the tube from the heating block. Add 900 μL of 2-Propanol into the tube and mix it with a vortex mixer.
6. Leave the tube at room temperature for 15 minutes.
7. Centrifuge the tube with 10,000 × g at room temperature for 15 minutes. A faint white pellet appears in the tube.
8. Remove supernatant from the tube by using a pipette or decanting carefully until the liquid volume in the tube is less than approximately 100 μL.

Notes :
・Be careful not to remove the pellet from the tube.
・Centrifuge the tube with 10,000 × g at room temperature for 15 minutes again if the pellet floats.
・In case that residual liquid remains on the tube wall, place the tube upside down on a paper filter to remove it.

9. Add 800 μL of "Washing Solution A, CP" into the tube and mix it by inversion mixing. Ensure that the pellet is detached from the tube wall.
10. Centrifuge the tube with 10,000 × g at room temperature for 5 minutes.
11. Remove supernatant from the tube by using a pipette or decanting carefully until the liquid volume in the tube is less than approximately 100 μL.
    Notes :
    ・Be careful not to remove the pellet from the tube.
    ・Centrifuge the tube with 10,000 × g at room temperature for 5 minutes again if the pellet floats.
    ・In case that residual liquid remains on the tube wall, place the tube upside down on a paper filter to remove it.
12. Add 1.5 mL of Solution II into the tube and mix it by inversion mixing.
13. Centrifuge the tube with 10,000 × g at room temperature for 5 minutes.
14. Remove as much supernatant as possible from the tube by using a pipette or decanting carefully.
    Notes :
    ・Be careful not to remove the pellet from the tube.
    ・Centrifuge the tube with 10,000 × g at room temperature for 5 minutes again if the pellet floats.
    ・In case that residual liquid remains on the tube wall, place the tube upside down on a paper filter to remove it.
15. Dry the pellet by warming it at 60℃ in a heating block, vacuum dryer or natural drying.
    Notes :
    ・Too dry pellet is difficult to be dissolved. Stop drying the pellet when it still is wet.
    ・Much residual liquid in the tube may cause low qPCR efficiency because Solution II contains ethanol, which can inhibit qPCR.
    ・The pellet contains DNA and glycogen.
16. Dissolve the pellet with sterile distilled water or buffer, and proceed with qPCR.

【Simplified Protocol】

This protocol is a DNA extraction method for purified proteins and serum samples that are relatively low in impurities.
Sample pretreatment is omitted and the number of washing step is one. This reduces the operating process and operating time.

<Preparation of Reagents>

1. Solution I (Simplified version)
   Add 6 mL of sterile distilled water, 1 mL of "Sodium N-Lauryl Sarcosinate Solution, CP" and 65 μL of "Glycogen Solution, CP" into the bottle of 26 mL of "Sodium Iodide Solution, CP". Mix it with a vortex mixer.
   Note : Solution I (Simplified version) can be stored at 2-10℃ in the dark for approximately 5 months.

2. Solution II
   Add 2 μL of "Glycogen Solution, CP" into the bottle of 40 mL of "Washing Solution B, CP". Mix it with a vortex mixer immediately.
   Notes :
   ・It does not affect quality of DNA extraction if white precipitates appear in Solution II.
   ・Solution II can be stored at 2-10℃ in the dark for approximately a week at least. If preparing a small volume of Solution II, use required amount of "Glycogen Solution, CP" and "Washing Solution B, CP". For example, add 1 μL of "Glycogen Solution, CP" into 20 mL of "Washing Solution B, CP" in a sterilized tube.

<DNA Extraction Procedure>

1. Dispense 100 μL of sample solution to a 2 mL or 1.5 mL blank centrifuge tube.

2. Add 300 μL of Solution I (Simplified version) into the tube and mix it with a vortex mixer.
3. Warm the tube at 60℃ for 15 minutes in a heating block.
4. Remove the tube from the heating block. Add 400 μL of 2-Propanol into the tube and mix it with a vortex mixer.
5. Leave the tube at room temperature for 15 minutes.
6. Centrifuge the tube with 10,000 × g at room temperature for 15 minutes. A faint white pellet appears in the tube.
7. Remove supernatant from the tube by using a pipette or decanting carefully until the liquid volume in the tube is less than approximately 100 μL.
   Notes :
   ・Be careful not to remove the pellet from the tube.
   ・Centrifuge the tube with 10,000 × g at room temperature for 15 minutes again if the pellet floats.
   ・In case that residual liquid remains on the tube wall, place the tube upside down on a paper filter to remove it.
8. Add 1 mL of Solution II into the tube and mix it by inversion mixing. Ensure that the pellet is detached from the tube wall.
9. Centrifuge the tube with 10,000 × g at room temperature for 5 minutes.
10. Remove as much supernatant as possible from the tube by using a pipette or decanting carefully.
    Notes :
    ・Be careful not to remove the pellet from the tube.
    ・Centrifuge the tube with 10,000 × g at room temperature for 5 minutes again if the pellet floats.
    ・In case that residual liquid remains on the tube wall, place the tube upside down on a paper filter to remove it.
11. Dry the pellet by warming it at 60℃ in a heating block, vacuum dryer or natural drying.
    Notes :
    ・Too dry pellet is difficult to be dissolved. Stop drying the pellet when it still is wet.
    ・Much residual liquid in the tube may cause low qPCR efficiency because Solution II contains ethanol, which can inhibit qPCR.
    ・The pellet contains DNA and glycogen.
12. Dissolve the pellet with sterile distilled water or buffer, and proceed with qPCR.

【FAQ】

Q. Peak wavelength appeared at 230 nm when extracted DNA concentration was measured by absorbance.
A. Sodium Iodide remains in the extracted DNA. Measure the amount of DNA by qPCR

Q. DNA recovery rate was too low.
A. A part of DNA pellet may have been removed at step 7 or 10. Remove the supernatant with a pipette carefully.

<Reference>
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FUJIFILM Wako Pure Chemical Corporation

1-2, Doshomachi 3-Chome, Chuo-Ku, Osaka 540-8605, Japan
Telephone : + 81-6-6203-3741
Facsimile : + 81-6-6201-5964

FUJIFILM Wako Chemicals (Hong Kong) Limited
Room 1111, 11/F, International Trade Centre, 11-19 Sha Tsui Road, Tsuen Wan, N.T., Hong Kong
Telephone : + 852-2799-9019
wkhk.info@fujifilm.com

FUJIFILM Wako (Guangzhou) Trading Corporation
Room 3003, 30/F., Dong Shan Plaza 69, Xian Lie Zhong Road, Guangzhou, 510095, China
Telephone : + 86-20-8732-6381(Guangzhou)
Telephone : + 86-21-6288-4751(Shanghai)
Telephone : + 86-10-6413-6388(Beijing)
wkgz.info@fujifilm.com

https://labchem-wako.fujifilm.com

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