BioChain®
www.biochain.com
Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

User's Manual and Instructions

uPure Urine DNA Extraction Kit

Catalog Number: K5011196

Storage Conditions
This kit is shipped at room temperature. Upon receipt, store Proteinase K at -20°C. Store all remaining components of this kit at room temperature.

Shelf Life
1 year from the date of receipt under proper storage conditions

Features
- Non-toxic chemicals
- High DNA recovery
- Short and Scalable Protocol
- Obtain either cfDNA from fluid portion or genomic DNA from pellet (both human and bacterial)
- Purified DNA is suitable for NGS, PCR, Bisulfite sequencing, etc

Description
BioChain's uPure Urine DNA Extraction kit allows for fast and efficient cell-free DNA (cfDNA) and/or genomic DNA extraction from human urine samples. The genomic DNA will be derived from a mix of pelleted bacterial cells and exfoliated human cells.

The DNA is eluted in EDTA-free buffer, allowing for immediate use in experiments where EDTA is not tolerated. EDTA may then be added separately for ideal long-term storage of the DNA. The magnetic bead-based extraction protocol is also ideally suited for automation using the KingFisher instrument. DNA extracted using this kit is suitable for PCR, qPCR, next generation sequencing (NGS), and other applications.

KingFisher Automation
The uPure Urine DNA Extraction Kit can be used to isolate genomic DNA from up to 96 urine pellet samples and/or cfDNA from up to 250 ml urine using the KingFisher™ Flex Magnetic Processor with 96- and/or 24- Deep Well Head, respectively. An alternative protocol below describes the use of the uPure kit with the KingFisher™ Flex Magnetic Processor.

Contents
This kit contains all necessary reagents for the isolation of genomic DNA from up to 96 urine pellets or cfDNA from up to 250 ml of urine.

Quality Control
Each component has been tested for purity and efficacy.

Important Notes
Starting Material: Fresh or preserved urine samples can be used with the DNA isolation protocol.

Quantification: DNA quantities are dependent on the donor and starting volume but are generally low for urine samples. Therefore, we recommend Qubit and/or qPCR quantification for accuracy.

Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

Equipment and Reagents to be Supplied by User

- Pipettes
- Vortex-Genie 2 or similar vortexing mixer*
- Magnet stand for molecular applications (e.g. DynaMag™-15 or DynaMag™-2)
- 1.5 ml non-stick Eppendorf tubes
- 15 ml or 50 ml conical tubes
- 100% EtOH (200 proof)
- Isopropanol

* Contact BioChain® Technical Service for additional recommendations for high throughput or automated mixing.

Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

Prior to Initial Use

Box 1 (cfDNA Extraction Reagents)

ULB and UW buffers are shipped as a concentrate. If precipitate is present in either solution, incubate solution at 37°C for 30 minutes with occasional shaking. Add the following user-supplied reagents to each bottle:

ULB Buffer
- Add 19 ml of fresh 100% ethanol (200 proof) to each bottle and mix by inverting gently

UW Buffer
- Add 51 ml of fresh 100% ethanol (200 proof) to each bottle and mix by inverting gently

- Prepare fresh 80% ethanol solution prior to each extraction

Box 2 (Genomic DNA Extraction Reagents)

BFC, FCW1, and FCW2 Buffers are shipped as a concentrate. If precipitate is present in any of these solutions, incubate solution at 37°C for 30 minutes with occasional shaking. Add the following user-supplied reagents to each bottle:

BFC Buffer
- Add 26.4 ml of isopropanol and mix by inverting gently

FCW1 Buffer
- Add 10.2 ml of 100% ethanol (200 proof) and mix by inverting gently

FCW2 Buffer
- Add 52.8 ml of 100% ethanol (200 proof) and mix by inverting gently

Once these alcohols are added, these buffers are stable for one year if stored properly.

The genomic DNA extraction protocol below was written assuming an initial sample volume of up to 5 ml urine. If using more volume, the reagents may need to be scaled depending on the pellet size.

Protocol

Sample Pre-processing

1. Centrifuge up to 5 ml of urine per tube at 16,000 x g for 10 minutes at 4°C
2. Carefully transfer the supernatant to a fresh tube without disturbing the pellet (leave behind ~50-100 µl of liquid). To isolate cfDNA from the supernatant, go to “Cell-Free DNA Extraction” protocol (sample may temporarily be stored at -80°C until ready for extraction)

   Note: If cfDNA is not needed, then the supernatant may be discarded.
3. Pipet pellet up and down 10 times and transfer to 1.5 ml Eppendorf tube.

4. Centrifuge at 8,000 rpm for 3 minutes and remove as much supernatant as possible from the pellet (sample may temporarily be stored at -80°C until ready for extraction)

Genomic DNA Extraction

Lysis/Binding

1. Add 400 µl of **UFC Buffer** to the pellet
2. Add 10 ul of **Proteinase K**
3. Pipet pellet up and down 10 times or vortex to mix and incubate at 56°C for 10 minutes
4. Vortex briefly to mix and incubate at 56°C for an additional 10 minutes
5. Spin tube briefly on a tabletop centrifuge to bring down solution from the lid of the tube and add 300 µl of **BFC buffer**
6. Add 5 µl of **Magnetic Bead Solution**

   Important: Mix beads well prior to adding. There should be no visible sedimentation at the bottom of the solution after mixing. Beads will settle quickly, so be sure to mix the magnetic bead solution after adding to every few samples. Failure to do so may result in inconsistent yields
7. Vortex vigorously for 10 minutes at room temperature

   * A vortexing mixer with a tube(s)-holder that allows for walk-away mixing will make this step easier.
8. Place tube onto a magnet stand and let sit for 3 minutes
9. While keeping the tube on the magnet stand, remove supernatant. Be careful not to remove magnetic particles
10. Tap magnet stand on bench 5 times and remove remaining supernatant

Wash Steps

11. Transfer tube to non-magnetic rack and add 200 µl of **FCW1 Buffer**
    Resuspend beads by vortexing for 15 seconds. Spin tube briefly and transfer back to magnet stand

    *Centrifuge steps are needed when using vortexing to resuspend beads. Only a brief spin is recommended to remove solution from tube lid. ALWAYS check to make sure the beads are at the bottom of the tube before spinning so that they don't become stuck on the side of the tubes and dry out. If beads are attached to the sides, flick the tube until beads are in solution
12. Allow beads to attach to magnet stand for 10-30 seconds
13. Remove as much supernatant as possible
14. Transfer tube to non-magnetic rack and add 200 µl of **FCW2 Buffer**
15. Resuspend beads by vortexing for 15 seconds. Spin tube briefly and transfer back to magnet stand

16. Allow beads to attach to magnet stand for 10-30 seconds
17. Remove as much supernatant as possible
18. Transfer tube to non-magnetic rack and add 200 µl of **FCW2 Buffer**
19. Resuspend beads by vortexing for 15 seconds. Spin tube briefly and transfer back to magnet stand
20. Allow beads to attach to magnet stand for 10-30 seconds
21. Remove as much supernatant as possible
22. Repeat steps 18-21 above once
23. Tap magnet stand on bench 5 times and remove residual supernatant as much as possible
24. Allow beads to dry for 3 minutes

Elution Step

25. Transfer tube to non-magnetic rack and add 50 µl of **FCE Buffer**
    Important:: A minimum of 50 µl of FCE Buffer is recommended to elute DNA to ensure optimal yields
26. Vortex vigorously for 10-15 seconds to loosen bead clumps
27. Incubate at 72°C for 5 minutes
28. Vortex vigorously for 10-15 seconds to loosen any remaining bead clumps
29. Spin tube briefly
30. Place tube on magnetic rack for 3 minutes
31. Transfer eluate* into a new 1.5 ml Eppendorf tube

*** Note:** To maximize the utilization of the final product in downstream applications, the Elution Buffer does not contain EDTA. If long-term storage of the DNA is desired, we recommend adding EDTA to a final volume of 1 mM (e.g. add 1 ul of 100 mM EDTA pH 8.0 stock solution for every 100 ul of elution volume).

Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

Cell-Free DNA Extraction

Before starting the protocol, determine the amount of urine to be used for extraction and calculate the amount of buffer and beads needed. Any amount from 1 ml to 10 ml of urine can be used. Due to the very low cfDNA yields in urine, we do not recommend starting with volumes less than 1 ml. Scale buffer and bead volumes accordingly using the table below.

Urine	Lysis/Binding Buffer	Isopropanol	Bead Solution	Tube(s) size
x (x=ml of urine)	1.25x	0.5x	0.008x	n/a
5 ml	6.25 ml	2.5 ml	40 µl	15 ml or 50ml*
10 ml	12.5 ml	5 ml	80 µl	50 ml

*Using a 50 ml tube(s) for 5 ml or more of urine is recommended over a 15 ml tube(s). While a 15 ml tube(s) will work it may lead to slightly lower yields

Lysis/Binding

1. Add the appropriate amount of urine to appropriately sized tube
2. Add 1.25 ml of **ULB Buffer** for every 1 ml of urine used
3. Add 8 µl of **Magnetic Bead Solution** for every 1 ml of urine

   Important: Mix beads well prior to adding. There should be no visible sedimentation at the bottom of the solution after mixing. Beads will settle quickly, so be sure to mix the magnetic bead solution after adding to every few samples. Failure to do so may result in inconsistent yields
4. Vortex or shake tube vigorously for 10 minutes at room temperature

   * To obtain high yields, ensure that urine/buffer solution is mixing vigorously in tube. A vortexing mixer with a tube(s)-holder that allows for walk-away mixing will make this easier.
5. Place tube into a magnet stand for 2 to 5 minutes, or until solution clears
6. While keeping the tube on the magnet stand, remove supernatant. Be careful not to remove magnetic particles
7. Keep tube on magnet stand for 1 minute, and remove residual supernatant

First Wash

8. Add 1000 µl of **UW Buffer** to lysis/binding tube
9. Resuspend beads by vortexing for 10 seconds or pipetting up and down 10 times
10. Transfer magnetic particle suspension into 1.5 ml micro tube on magnet stand

11. Allow beads to attach to magnet stand for 10-30 seconds
12. Pipet supernatant from 1.5 ml tube and use the supernatant to wash the lysis/binding tube
13. Transfer the rest of the magnetic particles in lysis/binding tube to the 1.5 ml tube
14. Keep tube on magnet stand for 10-30 seconds or until solution is clear
15. Remove as much buffer as possible using a 1000 µl pipette
16. Tap magnet stand on bench 5 times and remove remaining wash buffer with 200 µl pipette
17. Transfer tube to non-magnetic rack and add 1000 µl of **UW Buffer**
18. Resuspend beads by vortexing for 20 seconds or pipetting up and down 10 times
19. Centrifuge tube briefly

    *Centrifuge steps are needed when using vortexing to resuspend beads. Only a brief spin is recommended to remove solution from tube lid
20. Place tube on magnet stand for 10-30 seconds
21. Remove as much buffer as possible using a 1000 µl pipette
22. Tap magnet stand on bench 5 times and remove remaining wash buffer with 200 µl pipette

Second Wash

23. Transfer tube to non-magnetic rack and add 1000 µl of **80% EtOH**
24. Resuspend beads by vortexing for 20 seconds or pipetting up and down 10 times
25. Centrifuge tube briefly
26. Place on magnet stand for 10-30 seconds or until solution clears
27. Remove as much buffer as possible using a 1000 µl pipette
28. Tap magnet stand on bench 5 times and remove remaining EtOH with 200 µl pipette
29. Transfer tube to non-magnetic rack and add 1000 µl of **80% EtOH**
30. Resuspend beads by vortexing for 20 seconds or pipetting up and down 10 times
31. Centrifuge tube briefly
32. Place on magnet stand for 10-20 seconds
33. Remove as much EtOH as possible using a 1000 µl pipette and leave cap open
34. Tap magnet stand with tube on bench 5 times
35. Remove remaining EtOH with 200 µl pipette
36. Leave tube open on magnet stand for two minutes and then tap tube on bench 5 times and remove any remaining EtOH with 20 µl pipette
37. Allow magnetic particles to dry for an additional 1-3 minutes

    *Be careful to not over dry or beads may stick to tube

Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

Elution Step

38. Transfer microtube to non-magnetic rack and add desired volume of **UE Buffer**
    Important:: A minimum of 12.5 µl of UE1 Buffer per ml of urine is recommended to elute DNA to ensure optimal yields
39. Vortex or shake tube vigorously for 5 minutes
40. Centrifuge tube briefly
41. Place tube on magnetic rack for 10 to 30 seconds
42. Transfer eluate into a new 1.5 ml tube

Kit Components

uPure Urine DNA Extraction Kit, Box 1 of 2 (K5011196)

Item	Cat#	Amount	Storage
1. ULB Buffer	K5011196-1	3 x 95 ml	Room Temp
2. UW Buffer	K5011196-2	5 x 55 ml	Room Temp
3. UM Bead Solution	K5011196-3	5 x 1.33 ml	Room Temp
4. UE Buffer	K5011196-4	1 x 15 ml	Room Temp

uPure Urine DNA Extraction Kit, Box 2 of 2 (K5011196)

Item	Cat#	Amount	Storage
1. UFC Buffer	K5011196-5	40 ml	Room Temp
2. BFC Buffer	K5011196-6	6 ml	Room Temp
3. Proteinase K	K5011196-7	1 ml	-20°C
4. Magnetic Bead Solution	K5011196-8	500 µl	Room Temp
5. FCW1 Buffer	K5011196-9	10 ml	Room Temp
6. FCW2 Buffer	K5011196-10	12 ml	Room Temp
7. FCE Buffer	K5011196-11	15 ml	Room Temp

BioChain®
www.biochain.com
Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

uPure Urine DNA Extraction Kit

Isolation of cfDNA from 1 - 4 ml of urine sample using KingFisher™ Flex Magnetic Processor 24DW

Catalog Number: K5011196

Product Description

Biochain's new uPure Urine DNA Extraction Kit has been designed to isolate both circulating DNA and genomic DNA (bacterial and human) from human urine samples. The kit utilizes Silica coated magnetic bead technology allowing for scalability and easy automation. This guide describes the use of the uPure kit with the KingFisher™ Flex Magnetic Processor 24DW to process samples of 1 - 4 ml.

Kit Contents and Storage

uPure Urine DNA Extraction Kit Box 1 of 2 (K5011196)

Item	Amount	Storage
ULB Buffer	3 x 95 ml	Room Temp.
UW Buffer	5 x 55 ml	
UM Bead Solution	5 x 1.33 ml	
UE Buffer	1 x 15 ml	

Equipment and Reagents to be Supplied by User

Item	Source
Equipment	
Multi-channel micropipettors	Any
Adjustable Micropipettors	Any
Vortexer	Any
Magnetic Particle Processor	
KingFisher™ Flex Magnetic Particle Processor	Thermofisher 5400630
Magnetic Head	
24 Deep-Well Head for KingFisher™ Flex Magnetic Particle Processor	Thermofisher 24074440
Deep-Well Plates	
KingFisher™ Flex 24 deep well plate, sterile	Thermofisher 95040480
Tip Combs	
King Fisher Flex 24 Deep Well Tip Comb and Plate	Thermofisher 97002610

Item	Source
Consumables	
Aerosol-resistant pipette tips	Any
Nonstick, nuclease-free Microfuge tubes (1.5ml)	Any
MicroAmp™ Clear Adhesive Film	Any
Reagent Reservoirs	Any
Reagents	
Ethanol, 200 proof (Absolute)	Any
Isopropanol	Any

Download KingFisher™ Flex Program

1.On uPure Webpage scroll down to Manual Section.
2. Click uPure_3-4ml_Flex and/or uPure_1-2ml_Flex to download program to your computer
3. Refer to KingFisher™ Flex manual for instructions for installing program on the instrument

Important Notes

Starting Material: Both fresh and preserved urine can be used with the Cell-Free DNA isolation protocol.

Prior to Initial Use

The ULB and UW Buffers are shipped as a concentrate. If precipitate is present in either solution, incubate solution at 37°C for 30 minutes. 100% EtOH must be added to both solutions before the first use. Once EtOH is added, these buffers are stable for one year. Be sure to close the bottle tightly for long term storage.

- Add 19 ml of fresh 100% ethanol to each bottle of ULB Buffer and mix by inverting gently
- Add 51 ml of fresh 100% ethanol to each bottle of UW Buffer and mix by inverting gently
- Prepare fresh 80% ethanol solution prior to each extraction

Sample Pre-processing

1. Centrifuge fresh or preserved urine sample at 16,000 x g for 10 minutes at 4°C.
2. Transfer supernatant to fresh tube/bottle. Leave behind ~50-100 ul of solution to avoid carryover of the pellet.

   If only cfDNA extraction is desired, then the pellet may be discarded. Otherwise, the protocol "Isolation of genomic DNA using KingFisher™ Flex Magnetic Processor 96DW" should be followed for pellet pre-processing instructions.
3. Prepare the rest of the plates according to the next table.

Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

Plate Set up for 1 or 2 ml samples

Set up 24 Deep Well Plates by adding appropriate reagents according to table below

Plate ID	Plate Type	Plate Position	Reagent	Volume per well	
				1 ml	2 ml
Lysis/Binding Plate	24 DW Plate	1	ULB Buffer	1.25 ml	2.5 ml
			UM Bead Solution	8 µl	16 µl
			Isopropanol	0.5 ml	1 ml
Wash Plate 1	24 DW Plate	2	UW Buffer	1 ml	
Wash Plate 2	24 DW Plate	3	UW Buffer	1 ml	
Wash Plate 3	24 DW Plate	4	80% Ethanol	2 ml	
Wash Plate 4	24 DW Plate	5	80% Ethanol	1 ml	
Elution Plate	24 DW Plate	6	UE Buffer	50 µl	
Tip Comb	24 DW Plate	7	Place a 24 Deep-Well Tip Comb in Plate		

Plate Set up for 3 or 4 ml samples

Set up 24 Deep Well Plates by adding appropriate reagents according to table below

Plate ID	Plate Type	Plate Position	Reagent	Volume per well	
				3 ml	4 ml
Lysis/Binding Plate 1	24 DW Plate	1	ULB Buffer	1.875 ml	2.5 ml
			UM Bead Solution	12 µl	16 µl
			Isopropanol	0.75 ml	1 ml
Lysis/Binding Plate 2	24 DW Plate	2	ULB Buffer	1.875 ml	2.5 ml
			UM Bead Solution	12 µl	16 µl
			Isopropanol	0.75 ml	1 ml
Wash Plate 1	24 DW Plate	3	UW Buffer	1 ml	
Wash Plate 2	24 DW Plate	4	UW Buffer	1 ml	
Wash Plate 3	24 DW Plate	5	80% Ethanol	2 ml	
Wash Plate 4	24 DW Plate	6	80% Ethanol	1 ml	
Elution Plate	24 DW Plate	7	UE Buffer	50 µl	
Tip Comb	24 DW Plate	8	Place a 24 Deep-Well Tip Comb in Plate		

- Gently shake Lysis/Binding Plate(s) to mix the reagents
- If extracting cfDNA from a 1 or 2 ml sample add entire sample to a well on Lysis/Binding Plate
- If extracting cfDNA from a 3 or 4 ml sample add half of sample to a well on Lysis/Binding Plate 1 and the other half of sample to the same well position on Lysis/Binding Plate 2

Instrument Set up

- Place 24 Deep-Well magnetic head on to machine according to the user manual
- Select uPure_3-4ml_Flex on the instrument for 3 or 4 ml extractions or uPure_1-2ml_Flex for 1 or 2 ml extractions
- Start the run and follow on screen prompts to load processing plates in their respective positions
- At the end of the run remove elution plate from machine and cover plate or transfer eluate to new tubes

Isolated cfDNA is ready for immediate use or can be stored at -20°C

BioChain®
www.biochain.com
Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

uPure Urine DNA Extraction Kit

Isolation of genomic DNA using KingFisher™ Flex Magnetic Processor 96DW

Catalog Number: K5011196

Product Description

Biochain's new uPure Urine DNA Extraction Kit has been designed to isolate both circulating DNA and genomic DNA (bacterial and human) from human urine samples. The kit utilizes Silica coated magnetic bead technology allowing for scalability and easy automation. This guide describes the use of the uPure kit with the KingFisher™ Flex Magnetic Processor 96DW to process pellet samples from up to 5 ml of urine.

Kit Contents and Storage

uPure DNA Extraction Kit, Box 2 of 2 (K5011196)

Item	Amount	Storage
UFC Buffer	40 ml	Room Temp.
BFC Buffer	6 ml	
Magnetic Bead Solution	500 µl	
FCW1 Buffer	10 ml	
FCW2 Buffer	12 ml	
FCE Buffer	15 ml	
Proteinase K	1 ml	-20°C

Equipment and Reagents to be Supplied by User

Item	Source
Equipment	
Multi-channel micropipettors	Any
Adjustable Micropipettors	Any
Vortexor	Any
Magnetic Particle Processor	
KingFisher™ Flex Magnetic Particle Processor 96DW	Thermofisher 5400630
Deep-Well Plates	
96 Deep-Well Plates for KingFisher™ Flex Magnetic Particle Processor	Thermofisher 95040460
Standard Plates	
96 Standard Plates for KingFisher™ Flex Magnetic Particle Processor	Thermofisher 97002540
Tip Combs	
96 Deep-Well Tip Combs for KingFisher™ Flex Magnetic Particle	Thermofisher 97002534
Consumables	
Aerosol-resistant pipette tips	Any
Nonstick, RNase-free Microfuge tubes (1.5ml)	Any
MicroAmp™ Clear Adhesive Film	Any
Reagent Reservoirs	Any
Reagents	
Ethanol, 200 proof (Absolute)	Any
Isopropanol	Any

Download KingFisher™ Flex Program

1.On uPure Webpage scroll down to Manual Section.

2. Click uPure_Pellet_Flex to download program to your computer

3. Refer to KingFisher™ Flex manual for instructions for installing program on the instrument

Important Notes

Starting Material: Cell pellets that have been spun down from human urine samples

Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

Prior to Initial Use

BFC, FCW1, and FCW2 Buffers are shipped as a concentrate. If precipitate is present in any of the solutions, incubate solution at 37°C for 30 minutes with occasional shaking. Add the following user-supplied reagents to each bottle:

- **BFC Buffer**

  Add 26.4 ml of isopropanol and mix by inverting gently

- **FCW1 Buffer**

  Add 10.2 ml of 100% ethanol (200 proof) and mix by inverting gently

- **FCW2 Buffer**

  Add 52.8 ml of 100% ethanol (200 proof) and mix by inverting gently

Sample Pre-processing

1. Centrifuge up to 5 ml of fresh or preserved urine sample at 16,000 x g for 10 minutes at 4°C.
2. Transfer supernatant to fresh tube/bottle. Leave behind ~50-100 ul of solution to avoid carryover of the pellet.

   If only cfDNA extraction is desired, then the pellet may be discarded and the protocol "Isolation of cfDNA from 1 -4 ml urine sample using KingFisher™ Flex Magnetic Processor 24DW" should be followed. Likewise, if only genomic DNA from the pellet is desired, this supernatant may be discarded.

3. Remove all of the supernatant from the pellet.

   Alternatively, pipet the pellet up/down and transfer to a fresh 1.5 ml Eppendorf tube.

   Centrifuge the tube at 8,000 rpm for 2 minutes and discard supernatant.

4. Add 400 µl of UFC buffer to the pellet, pipet up/down, and transfer the entire solution to a well on a 96-Deep-Well Plate.
5. Prepare the rest of the plates according to the next table.

Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

Plate Set up (for Pellet)
Set up 96 Deep Well Plates by adding appropriate reagents according to table below

Plate ID	Plate Type	Plate Position on Instrument	Reagent	Volume per well
Lysis/Binding Plate	96 Deep-Well Plate	1	UFC with Pellet	400 µl
			Proteinase K	10 µl
Wash Plate 1	96 Deep-Well Plate	2	FCW1 Buffer	200 µl
Wash Plate 2	96 Deep-Well Plate	3	FCW2 Buffer	200 µl
Wash Plate 3	96 Deep-Well Plate	4	FCW2 Buffer	200 µl
Wash Plate 4	96 Deep-Well Plate	5	FCW2 Buffer	200 µl
Elution Plate	96 Standard Plate	6	FCE Buffer	50 µl
Tip Comb	96 Deep-Well Plate	7	Place a 96 Deep-Well Tip Comb in Plate	

Instrument Set up

- Place 96 well Deep-Well magnetic head on to machine, and select uPure_Pellet_Flex on the instrument
- Start the run and follow on screen prompts to load processing plates in their respective positions
- 20 minutes after run starts a dispense step will prompt the user to add BFC Buffer and Magnetic Bead solution to Lysis/Binding Plate

Reagent	Volume per Well
BFC Buffer	300 µl
Magnetic Bead Solution	5 µl

- Place plate back into machine and press Start to continue protocol
- After program run ends isolated DNA is ready for immediate use

*** Note:** To maximize the utilization of the final product in downstream applications, the Elution Buffer does not contain EDTA. If long-term storage of the DNA is desired, we recommend adding EDTA to a final volume of 1 mM (e.g. add 1 ul of 100 mM EDTA pH 8.0 stock solution for every 100 ul of elution volume).