Real Genomics

Store at room temperature 15°C~25°C

HiYield™ Gel/PCR Fragments Extraction Kit
Mini Prep DNA Extraction
96-well PCR Clean Up Kit
Centrifuge/Vacuum System
Protocol Book

■ YDF100 // YDF300

■ YDF96B-2 // YDF96B-4 // YDF96B-10

Ver. 3.0


Real Genomics

Precautions

I) Handling Requirements

- *Do not use a kit after its expiration date has passed.*
- *Some reagents contain the hazardous compounds guanidine thiocyanate or guanidine hydrochloride . Do not let these reagents touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water. If you spill the reagents, dilute the spill with water before wiping it up.*
- *Do not allow reagents containing guanidine thiocyanate to mix with sodium hypochlorite solution or strong acids. This mixture can produce a highly toxic gas.*

II) Laboratory Procedures

- *Handle all samples and the resulting waste as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator has to optimize pathogen inactivation by the Lysis Buffer or take appropriate measures according to local safety regulations. RBC Bioscience does not warrant that samples treated with Lysis Buffer are completely inactivated and non-infectious. After sample processing is completed, remove and autoclave all disposable plastics, if you worked with potentially infectious sample material.*
- *Do not eat, drink or smoke in the laboratory work area.*
- *Wear protective disposable gloves, laboratory coats and eye protection when handling samples and kit reagents.*
- *Do not use sharp or pointed objects when working with the reagent cartridge, in order to prevent damage of the sealing foil and loss of reagent.*
- *Do not contaminate the reagents with bacteria, virus, or ribonuclease. Use disposable pipettes and RNase-free pipette tips only to remove aliquots from reagent bottles. Use the general precautions described in the literature.*
- *Wash hands thoroughly after handling samples and test reagents.*

III) Waste Handling

- *Discard unused reagents and waste in accordance with country, federal,state and local regulations.*

CONTENTS

HiYield™

Mini prep DNA Extraction

HiYield™ Gel/PCR DNA Fragments Extraction Kit

Cat.No. YDF100//YDF300

Kit Contents

Cat.No. YDF100

100 mini preps / kit

DF Buffer(B049)	80ml
Wash Buffer (concentrated)*	25ml
Elution Buffer	10ml
2ml Collection Tube	100pcs
DF Cloumn	100pcs

Cat.No. YDF300

300 mini preps / kit

DF Buffer(B050)	200ml
Wash Buffer (concentrated)**	40ml
Elution Buffer	30ml
2ml Collection Tube	300pcs
DF Cloumn	300pcs

Additional DF Buffer (cat.No. B049/B050) can be purchased separately.

Sample : 200 mg Gel / 100 µl PCR Solution
Yields: Gel 70 - 80 % / PCR Recovery 80 - 90 %
Eective Binding Capacity: Appro x 10 µg
Eective Primer Removal : < 25 bp

Operation Time: 20 mins
Elution Volume: 20-50µl
Seq. Cut-Off: 50bp - 10kb

* Add 100ml ethanol (96-100%) to Wash Buffer prior to initial use.
** Add 160ml ethanol (96-100%) to Wash Buffer prior to initial use.

Description

The HiYield ™ Gel / PCR DNA Fragments Extraction Kit is designed to recover or concentrate DNA fragments (50bp - 10Kb) from agarose gels, PCR or other enzymatic reactions. The unique dual purpose application and high yield DNA / minicolumn make this kit exceptional value. The method uses a chaotropic salt , guanidine thiocyanate to dissolve agarose gel and denature enzymes. DNA fragments in chaotropic salt solution bind to the glass fiber matrix of the spin column. Following washing off contaminants, the purified DNA fragments are eluted by addition of low salt elution buffer or water. Salts, enzymes and unincorporated nucleotides are effectively removed from reaction mixtures without phenol extraction or alcohol precipitation .

Quality Control

The quality of HiYield™ Gel / PCR DNA Fragments Extraction Kit is tested on a lot - to - lot basis . The efficiency of DNA recovery is tested by isolation of DNA fragments of various sizes from either aqueous solution or agarose gel .
The purified DNA is checked by agarose gel analysis.

Reference

Vogelste in , B. , and Gillespie , D. (1979) Proc . Natl. Acad. Sci. USA 76 , 615.

Note

* *DF Buffer contains guanidine thiocyanante which is harmful and an irritant agent. During operation , always wear a lab coat , disposable gloves, and protective goggles.*

Gel Extraction Protocol

Gel Dissociaton

1. Excise the agarose gel slice containing relevant DNA Fragments and remove extra agarose to minimize gel slice.
2. Transfer up to 300mg of the gel slice into a microcentrifuge tube (not provided).
3. Add 500 µl of DF Buffer to the sample and mix by vortexing.
4. Incubate at 55°C for 10-15 minutes until the gel slice has been completely dissolved. During incubation, invert the tube every 2-3 mins.

For best efficiency and more precisely at excising the agarose gel slice, RBC Gel Cutter is available. Please contact your local RBC products supplier for further information.

DNA Binding

5. Place a DF Column into a Collection Tube.
6. Apply 800µl of the sample mixture from previous step into the DF Column.
7. Centrifuge at max. 10,000 xg (13,000 rpm) for 30 seconds.
8. Discard the flow-through and place the DF Column back in the Collection Tube.
9. If the sample mixture is more than 800 µl, repeat this DNA Binding Step.

Wash

10.Add 600µl Wash Buffer (ethanol added) to the DF Column.
11.Centrifuge at max. 10,000 xg (13,000 rpm) for 30 seconds.
12.Discard the flow-through and place the DF Column back into the Collection Tube.

> ***For TAE gels, proceed to step 13. For TBE gels, repeat Wash Steps 10-12.***
> ***Boric Acid is difficult to remove and can affect downstream applications, therefore double wash is recommended.***

13.Centrifuge again for 2 minutes at max 10,000 xg (13,000 rpm) to dry the column matrix.

DNA Elution

14.Transfer dried DF Column into a new microcentrifuge tube (not provided).
15.Add 20-50µl Elution Buffer or water to the center of the column matrix.
16.Allow to stand for 2 minutes until Elution Buffer or water is absorbed by the matrix.
17.Centrifuge for 2 minutes at full speed to elute purified DNA.

PCR Clean Up Protocol

Sample preparation

1. Transfer up to 100 µl reaction product to a microcentrifuge tube (not provided).
2. Add 5 volume of DF Buffer to 1 volume of the sample and mix by vortexing.

DNA Binding

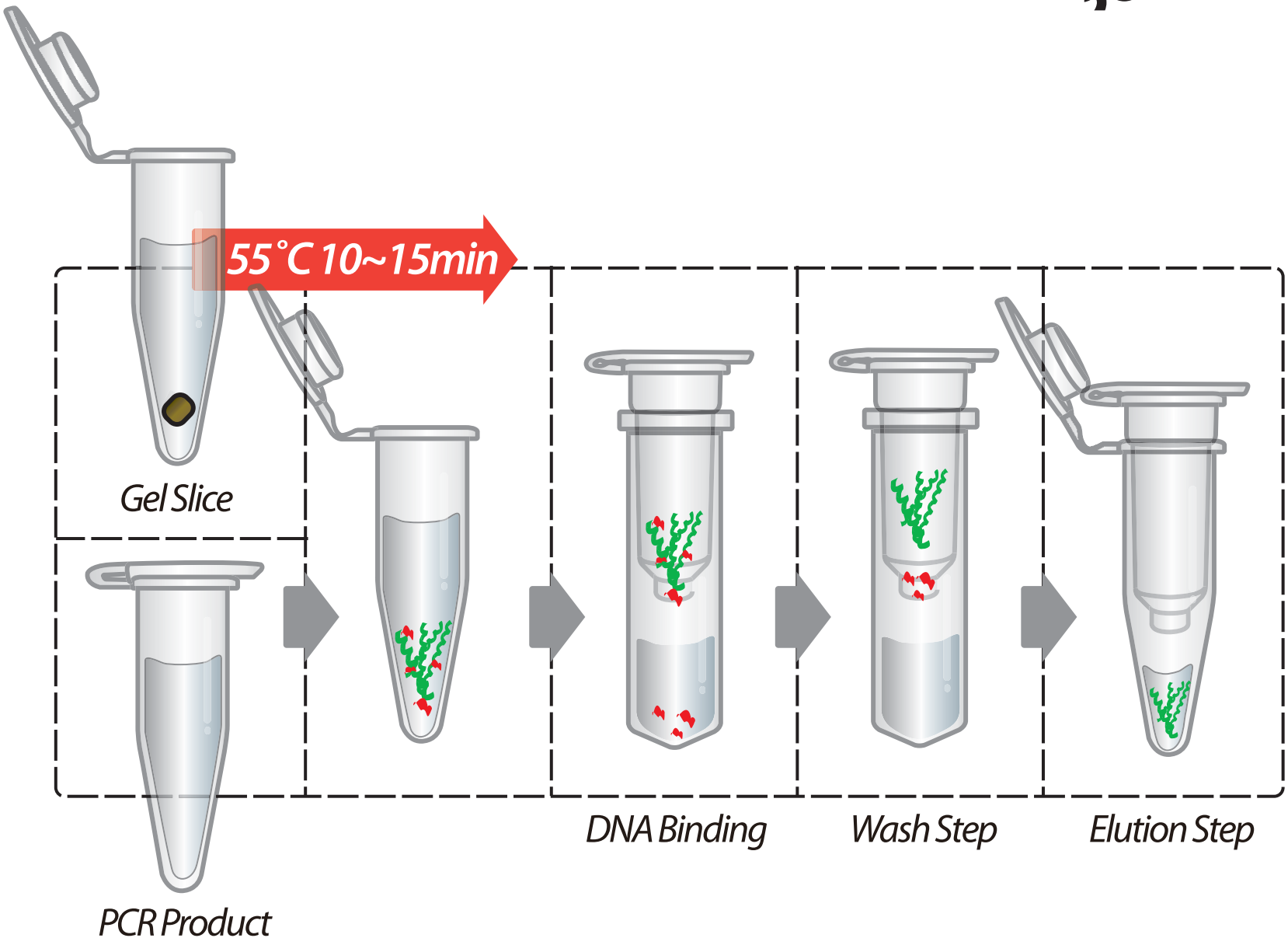
3. Place a DF Column into a Collection Tube.
4. Apply the sample mixture from previous step into the DF Column.
5. Centrifuge at max. 10,000 xg (13,000 rpm) for 30 seconds.
6. Discard the flow-through and place the DF Column back in the Collection Tube.

Wash

7. Add 600µl of Wash Buffer (ethanol added) to the DF Column.
8. Centrifuge at max. 10,000 xg (13,000 rpm) for 30 seconds.
9. Discard the flow-through and place the DF Column back in the Collection Tube.
10. Centrifuge again for 2 minutes at max. 10,000 xg (13,000 rpm) to dry the column matrix.

DNA Elition

11. Transfer dried DF Column into a new microcentrifuge tube (not provided).
12. Add 20-50 µl Elution Buffer or water to the center of the column matrix.
13. Allow to stand for 2 minutes until Elution Buffer or water is absorbed by the matrix.
14. Centrifuge for 2 minutes at max. 10,000 xg (13,000 rpm) to elute purified DNA.


55˚C 10~15min
Gel Slice
PCR Product
DNA Binding
Wash Step
Elution Step

Trouble Shooting

Problem	Possible Reason
Low recovery of DNA fragment	Size of DNA fragment is more than 5 kb
	Ineffective DNA elution
	Incomplete DNA elution
	TAE or TBE buffer is repeatedly used or of in correct pH
	Overloaded column with agarose
Poor performance in downstream applications	Eluted DNA carries salt residues
Non - Specitic DNA fragment appears in eluted DNA	DNA fragment is denatured and becomes single - stranded
	Scalpel or razor blade used to excise gel is contaminated
Gel slice diffcult to dissolve	Used high percentage agarose gel (> 2 . 5 %) (not recommended)
	Gel slice is too big (> 300 mg)

Solution
Use elution solution preheated to 60° C
DNA elution may be in effective in acidic conditions. Optimal elution pH is between 7.0 - 8.5
Make sure mi n . 30 µl is applied and Elution Buffer is applied to center of membrane . Allow time for full absorption to membrane prior to centrifugation.
Repeated use of TAE/TBE buffers will cause pH to increase. Use fresh TAE/TBE buffer.
Higher recovery is attained when smaller amounts of agarose are present. Minimize the size of the gel slice. If > 300mg , split sample and use another column.
Wash the column twice with Wash Buffer
To re - anneal ss DN A , incubate tube at 95° C and cool slowly at room temp.
Use a new or clean scalpel or razor blade to excise the gel
Add double volume of buffer for gels >2 . 5 % . Incubate with mixing every 1 - 2 minutes until complete dissolution .
Use more than one column for gel slice > 300mg

Centrifuge/Vacuum System

96-well PCR Clean Up Kit

Cat.No. YDF96B-2//YDF96B-4//YDF96B-10

Kit Contents

Cat.No. YDF96B-2
Validation Kit

0.35ml DNA Binding Plate	2 Plates
96-well 2ml Sample Plate	4 Plates
96-well PCR Plate	2 Plates
Sealing Film	5 sheets
Binding Buffer	65ml
Wash Buffer*	25ml
Elution Buffer	30 ml

Cat.No. YDF96B-4

0.35ml DNA Binding Plate	4 Plates
96-well 2ml Sample Plate	8 Plates
96-well PCR Plate	4 Plates
Sealing Film	10 sheets
Binding Buffer	125ml
Wash Buffer*	25ml X 2 bots
Elution Buffer	30 ml

Cat.No. YDF96B-10

0.35ml DNA Binding Plate	10 Plates
96-well 2ml Sample Plate	20 Plates
96-well PCR Plate	10 Plates
Sealing Film	25 sheets
Binding Buffer	160ml
Wash Buffer*	25ml X 5 bots
Elution Buffer	60 ml

Validation kit includes all components for testing centrifuge and vacuum protocol system.
Final contents will differ depending on user preference for centrifuge or vacuum protocol.

* Add 4 times volume of Ethanol (96-100%) to 1 volume of Wash Buffer prior to initial use.

Description

RBC Real Genomics 96-well PCR Clean Up Kit is based on a reliable convenient silica format and is compatible with both centrifuge and vacuum protocols. It contains all the necessary plates, buffers and accessories for easy use straight from the box. Using RBC Real Genomics 96-well PCR Clean Up Kit, as many as 96 amplification reactions can be processed in parallel in 25 minutes. The high quality silica membrane DNA filter plate ensures low well to well and batch to batch variability.

Principle

RBC Real Genomic 96-well PCR Clean Up Kit has the selective binding properties of a unique silica-gel membrane. The binding buffer is optimized for the efficient recovery of single and double stranded DNA fragments from 100bp to 10kb directly from amplification reactions, and for the quantitative removal of primers under 40 bases and incorporated nucleotides.

The RBC YDF96B system is ideally suited for cleaning up DNA from many applications, such as PCR, cDNA synthesis reactions, or ligation reactions.

Centrifuge

Any Medium/High Speed Centrifuge (Beckmann/Eppendorf/Qiagen/Sigma) equipped with 2 x 96 Plate Rotor.

Vacuum System

RBC 96 Vacuum Manifold or any standard 96/384 microplate vacuum manifold. The vacuum protocol is ideal for benchtop operation and a lower cost solution than centrifuging. However, due to differences in vacuum pressure between various manifold, vacuum systems and samples, it may be neccessary to optimise this step. RBC recommend a vacuum pressure starting from 10 inches Hg up to maximum 20 inches Hg to ensure all solution passes through each well. In practice all the wells should pass through solution within 3-5 minutes, the yields will show little variation even if some wells pass the solution through at varying speeds (some wells may process very rapidly). Maintain vacuum until the solution fully passes through each well.

Centrifuge Protocol

Sample Preparation

1. Add 5 times volume of Binding Buffer to the sample and mix by pipetting.
    a. For PCR sample less than 50µl, add Binding Buffer direcly to original PCR tube.
    b. For PCR sample larger than 50 µl, transfer PCR sample to a 2ml Collection Plate then add 5 times volume of Binding Buffer to each well.

DNA Binding

2. Place a 0.35ml DNA Binding Plate in a 2 ml Sample Plate.

This whole unit must be placed in the centrifuge, the DNA Binding Plate requires the rigid 2ml Sample Plate for each centrifugation step. The DNA Binding Plate must be sealed with sealing tape or provided Sealing Film for each centrifugation step. Sealing tape or Sealing Film may be reused if required.

3. Transfer the PCR mixture into the 0.35ml DNA Binding Plate.

For mixture volumes larger than the capacity of the DNA Binding Plate, repeat DNA Binding steps until all the mixture has been transferred to the membrane.

4. Seal with provided Sealing Film.
5. Centrifuge at 3,500 rpm for 5 min.
6. Discard the flow-through and return the 0.35ml DNA Binding Plate to the 2ml Sample Plate.

Wash

7. Add 300 µl of Wash Buffer (ethanol added) to each well of the 0.35ml DNA Binding Plate.
8. Centrifuge at 3,500 rpm for 5 min.
9. Discard the flow-through and return the DNA Binding Plate to the 2ml Sample Plate.
10. Add 300 µl of Wash Buffer (ethanol added) to each well of the 0.35ml DNA Binding Plate to wash again.
11. Centrifuge at 3,500 rpm for 5 min.
12. Centrifuge for another 10 minutes (or incubate at 60℃ for 5-10 minutes) to remove ethanol residue.

DNA Elution

Standard elution volume is 100 µl. If higher DNA concentration is required, reduce the Elution Buffer (30-50 µl) to increase DNA concentration.

13. Transfer the 0.35ml DNA Binding Plate to a clean 2 ml Sample Plate.
14. Add 50-100 µl Elution Buffer or ddH_2O (pH8.0-8.5) to the center of each well of the 0.35ml DNA Binding Plate.
15. Allow to stand for 3 minutes until the Elution Buffer or water has been absorbed by the matrix.
16. Centrifuge at 3,500 rpm for 5 min to elute purified DNA into a clean 2 ml Sample Plate.
17. Seal with Sealing Film or parafilm sealed lid and store at 4℃. For long term storage, store at -20℃.

Vacuum Protocol

Sample Preparation

1. Add 5 times volume of Binding Buffer to the sample and mix by pipetting.
    a. For PCR sample less than 50µl, add Binding Buffer directly to original PCR tube.
    b. For PCR sample larger than 50 µl, transfer PCR sample to a 2ml Sample Plate then add 5 times volume of Binding Buffer to each well.

DNA Binding

2. Place a 0.35ml DNA Binding Plate on top of the vacuum manifold. Place a waste tray or the provided 2ml Sample/ Collection Plate underneath to collect waste.
3. Transfer the PCR mixture into the 0.35ml DNA Binding Plate.

    For mixture volumes larger than the capacity of the DNA Binding Plate, repeat DNA Binding steps until all the mixture has been transferred to the membrane.

4. Place the 0.35ml DNA Binding Plate on top of vacuum manifold tightly.
5. Set vacuum manifold to 10-20 inches Hg for 3-5 minutes until wells have emptied.

    Refer to vacuum notes above for detailed instructions.

6. Turn off the vacuum and apply the rest of the mixture to each well of the DNA Binding Plate.
7. Set vacuum to 10-20 inches Hg for 3-5 minutes until wells have emptied.

Wash

8. Add 300 µl of Wash Buffer (ethanol added) to each well of the 0.35ml DNA Binding Plate.
9. Set vacuum to 10-20 inches Hg for 3-5 minutes until wells have emptied.
10. Add 300 µl of Wash Buffer (ethanol added) to each well of the 0.35ml DNA Binding Plate to wash again.
11. Set vacuum to 10-20 inches Hg for 3-5 minutes until wells have emptied.
12. Set vacuum for another 10 minutes (or incubate at 60℃ for 5-10 minutes) to remove ethanol residue.

DNA Elution

Standard elution volume is 100 µl. If higher DNA concentration is required, reduce the Elution Buffer (30-50 µl) to increase DNA concentration.

13. Place the 0.35ml DNA Binding Plate on the top of the vacuum manifold. Place the provided 96-well PCR Plate on top of the 2ml Sample Plate as support plate, then both placed inside the vacuum manifold. The 0.35ml DNA Binding plate will elute directly into the 96-well PCR Plate, which are in close proximity.
14. Add 50-100 µl Elution Buffer or ddH_2O (pH8.0-8.5) to the center of each well of the 0.35ml DNA Binding Plate.
15. Allow to stand for 3 minutes until the Elution Buffer or water has been absorbed by the matrix.
16. Set vacuum to 10-20 inches Hg for 1-2 minutes until wells have fully emptied and all elute is in the 96-well PCR Plate.
17. Seal with Sealing Film or parafilm sealed lid and store at 4℃. For long term storage, store at -20℃.


Sample Process
Bind
Wash –> Elute
Seal and Store

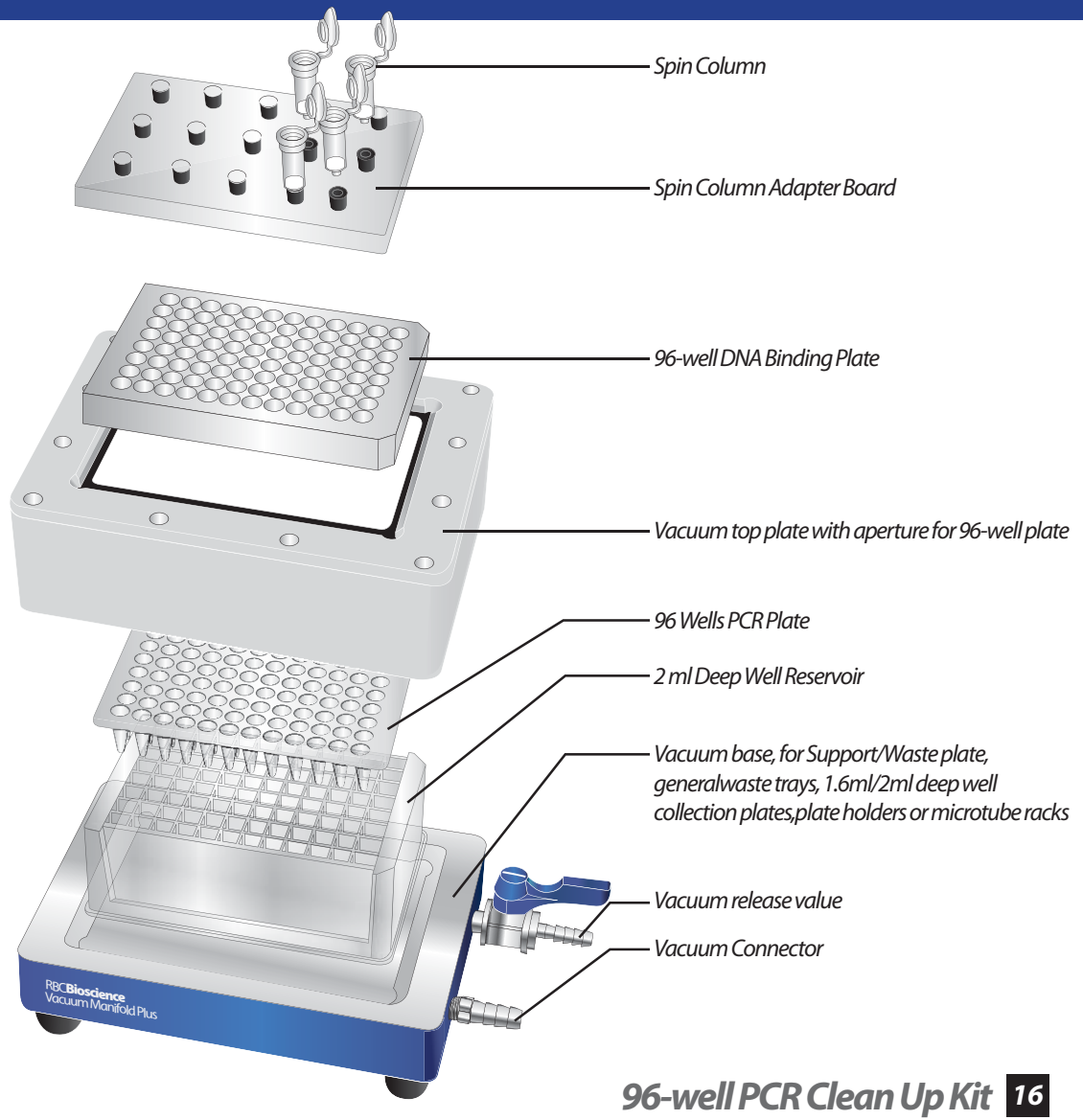
Multi Well Vacuum Manifold Plus
Cat.No. YVM96P

Multi Well Vacuum Manifold Plus

The multi well plate vacuum manifold is an aluminum manifold that has been designed and optimized for the vacuum

filtration of the 96-well filter plates.

* *Comes complete with the necessary O-ring and gasket. The control block includes the vacuum pressure gauge, vacuum metering valve, vacuum release valve, and the 1/4 inch hose barb for vacuum line attachment.*
* *Included with the vacuum manifold unit is a spacer block designed to accommodate standard 350µl receiver plates. The spacer block has been optimized to reduce the space between the receiver plate and the filter plate during vacuum filtration.*



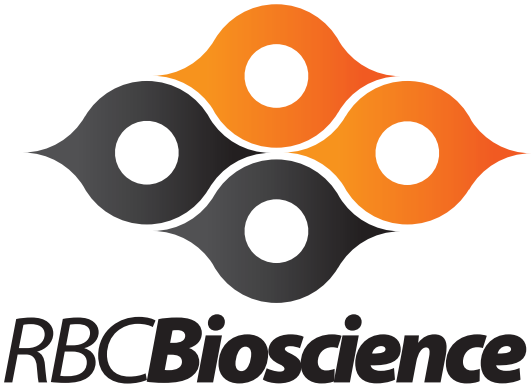
Trouble Shooting

Low Yield from DNA Elution	1. User utilized low pH elution buffer : Use provided Elution Buffer, alkaline elution buffer or ddH_20 (pH > 7.0). 2. Incorrect addition of ethanol : Add ethanol according to instructions, record on buffer bottle, after addition close buffer lid tightly. 3. High pressure vacuum pump: Lower pressure to 10 inches Hg to increase DNA binding time. 4. Insufficient incubation time for lysis: Incubate for at least 20 minutes for proteinase K complete digestion. 5. Insufficient mixing of lysis buffer , proteinase K and sample: Shake plate vigorously for 20 secs to ensure mixing. 6. DNA binding plate and Elution Buffer at room temperature for elution step: Heat both in incubator to 60°C prior to elute. 7. Add additional elution buffer step for low sample amounts e.g. extra 100 µl Elution buffer .
Eluate/Well Filter is slightly disclosured	1. Incomplete lysis of blood: Shake plate vigorously for 20 secs to ensure mixing of all reagents. 2. Incorrect washing procedure: Check ethanol was added correctly to wash buffer and wash buffer steps. Add wash step. 3. Overloaded column with sample: Split samples and process in two wells.
Cross Contamination	1. Well to well contamination observed: Insert tip ends to bottom of wells.Fix silicon cap/ tape f irmly. 2. Outside contamination: Operate using sterile conditions and equipment. Wear gloves at all times.

Poor performance of downstream application	***1. DNA yield too low for application: Increase amount by splitting into two wells for lysis step.*** ***2. DNA concentration is too low : Concentrate DNA by precipitation or vacuum.*** ***3. Contamination in eluate: Increase eluate amount to 200 µl or more and check contamination prevention procedure.*** ***4. RNA interfering with enzymatic procedures: Add RNase to degrade after Step 1 of Cell Lysis.***

Real Genomics	*96-well Extraction Range*	*Method*
YPD96B	*96-well Plasmid Kit*	*Silica Membrane*
YDF96B	*96-well PCR Clean Up Kit*	*Silica Membrane*
YVN96B	*96-well Viral Nucleic Acid Extraction Kit*	*Silica Membrane*
RBP96B	*96-well Genomic DNA Extraction (Blood) Kit*	*Silica Membrane*

For a complete HT solution including all neccessary reagents and buffers trust the Real Genomics 96-well range. Range from 2 plates (Validation), 4 plates , 10 plates, upwards.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