omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® DNA/RNA Kit

R6731-00	5 preps
R6731-01	50 preps

Manual Date: November 2018
Revision Number: v3.0

For Research Use Only

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E.Z.N.A.® DNA/RNA Kit

Table of Contents

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Introduction and Overview

E.Z.N.A.® DNA/RNA Kit provides a rapid and easy method for the isolation of total RNA and genomic DNA from cultured cells and animal tissues. The kit allows single or simultaneous processing of multiple samples in less than 40 minutes. Normally, 1 x 10^6 - 1 x 10^7 eukaryotic cells or 25-30 mg tissue can be used in a single experiment. There is no need for phenol/chloroform extractions, and time-consuming steps such as CsCl gradient ultracentrifugation and precipitation with isopropanol or LiCl are eliminated.

RNA purified using the E.Z.N.A.® DNA/RNA method is ready for applications such as RT-PCR*, Northern blotting, poly A+ RNA (mRNA) purification, nuclease protection, and *in vitro* translation.

The E.Z.N.A.® DNA/RNA Kit combines the reversible binding properties of HiBind® RNA technology with a specially designed buffer system which binds DNA to a DNA column before RNA isolation. Samples are first lysed and homogenized in a specially designed denaturing buffer (GTC Lysis Buffer), which immediately inhibits the activity of RNase and DNase. The lyste is then passed through a HiBind® DNA Mini Column which selectively binds genomic DNA. After two quick wash steps, the purified DNA is eluted. The filtered lysate from the HiBind® DNA Mini Column is combined with ethanol to create proper RNA binding conditions before being transferred to the HiBind® RNA Mini Column. After two wash steps, purified RNA is eluted with Nuclease-free Water.

New in this Edition:

November 2018:
- DEPC Water has been replaced with Nuclease-free Water. DEPC Water is no longer provided in this kit.
- PR032 (DEPC Water, 100 mL) has been discontinued and is no longer available to purchase.

June 2015:
- This manual has been edited for content and redesigned to enhance user readability.
- HB Buffer has been replaced by HBC Buffer. Isopropanol is required and supplied by the user.

Kit Contents

Product	R6731-00	R6731-01
Purifications	5	50
HiBind® RNA Mini Columns	5	50
HiBind® DNA Mini Columns	5	50
2 mL Collection Tubes	20	200
GTC Lysis Buffer	5 mL	40 mL
RNA Wash Buffer I	5 mL	30 mL
RNA Wash Buffer II	2 mL	12 mL
HBC Buffer	4 mL	25 mL
DNA Wash Buffer	1.5 mL	15 mL
Nuclease-free Water	2 mL	30 mL
Elution Buffer	2 mL	15 mL
User Manual	✓	✓

Storage and Stability

All of the E.Z.N.A® DNA/RNA Kit components are guaranteed for at least 12 months from the date of purchase when stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in GTC Lysis Buffer. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Preparing Reagents

1. Dilute RNA Wash Buffer II with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
R6731-00	8 mL
R6731-01	48 mL

2. Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
R6731-00	6 mL
R6731-01	60 mL

3. Dilute HBC Buffer with 100% isopropanol as follows and store at room temperature.

Kit	100% Isopropanol to be Added
R6731-00	1.6 mL
R6731-01	10 mL

4. Add 20 µL 2-mercaptoethanol (β-mercaptoethanol) per 1 mL GTC Lysis Buffer. This mixture can be stored for 4 weeks at room temperature.

Important Notes

Please take a few minutes to read this booklet in its entirety to become familiar with the procedures. Prepare all materials required before starting to minimize RNA degradation.

- Whenever working with RNA, always wear gloves to minimize RNase contamination. Use only clean RNase-free disposable plastic pipette tips when using the supplied reagents.

- Equilibrate samples and reagents to room temperature before beginning this protocol. All steps should be carried out at room temperature unless otherwise noted. Work quickly, but carefully.

- Prepare all materials required before starting the procedure to minimize RNA degradation.

- Carefully apply the sample or solution to the center of the HiBind® RNA Mini Columns. Avoid touching the membrane with pipet tips.

- 2-mercaptoethanol is key in denaturing RNases and can be added to an aliquot of GTC Lysis Buffer before use. Add 20 µL 2-mercaptoethanol per 1 mL GTC Lysis Buffer. This mixture can be stored for 4 weeks at room temperature.

Homogenization Techniques

Efficient sample disruption and homogenization is essential for successful total RNA isolation. Cell wall and plasma membrane disruption is necessary for the release of RNA from the sample and homogenization is necessary to reduce the viscosity of the lysates. Homogenization shears genomic DNA and other high-molecular-weight cell components creating a homogeneous lysate. Incomplete homogenization can cause the HiBind® RNA Mini Column to clog resulting in low or no yield.

Liquid Nitrogen Method

1. Wear appropriate gloves and take great care when working with liquid nitrogen.

2. Excise tissue and promptly freeze in a small volume of liquid nitrogen.

3. Grind tissue with a ceramic mortar and pestle under approximately 10 mL liquid nitrogen.

4. Pour the suspension into a pre-cooled 15 mL polypropylene tube.

   Note: Unless the tube is pre-cooled in liquid nitrogen, the suspension will boil vigorously and may cause loss of tissue.

5. Allow the liquid nitrogen to completely evaporate and add GTC Lysis Buffer.

6. Proceed to one of the homogenization steps below.

Homogenization - Choose one method below

1. Homogenizer Spin Columns and 2 mL Collection Tubes (HCR001, HCR003 and SSI-1370-00)

   - Load the lysate into a homogenizer spin column pre-inserted into a 2 mL Collection Tube (not provided).

   - Spin for two minutes at maximum speed in a microcentrifuge in order to collect homogenized lysate.

   - Proceed to Step 1 of the "Cultured Cell Protocol" on Page 8 or to Step 1 of the "Animal Tissue Protocol" on Page 15.

2. Syringe and Needle

   - Shear high-molecular-weight DNA by passing the lysate through a narrow needle (19-21 gauge) 5-10 times.

   - Proceed to Step 1 of the "Cultured Cell Protocol" on Page 8 or to Step 1 of the "Animal Tissue Protocol" on Page 15.

Rotor-Stator Homogenizer: Sample Disruption and Homogenization

Using a rotor-stator homogenizer for sample disruption and homogenization can simultaneously disrupt and homogenize most samples. The process usually takes less than a minute depending on sample type. Many rotor-stator homogenizers operate with differently sized probes or generators that allow sample processing in 50 mL tubes.

Bead Milling: Sample Disruption and Homogenization

By using bead milling, cells and tissue can be disrupted and homogenized by rapid agitation in the presence of glass beads and a lysis buffer. The optimal size of glass beads to use for RNA isolation are 0.5 mm for yeast/unicellular cells and 4-8 mm for animal tissue samples.

Syringe Needle: Sample Disruption and Homogenization

High-molecular-weight DNA is responsible for the viscosity of cell lysates and can be shredded by passing the sample 10-20 times through a narrow gauge needle (19-21 G).

E.Z.N.A.® DNA/RNA Kit - Cultured Cell Protocol

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 14,000 x *g*
- 70% ethanol
- 100% ethanol
- 100% isopropanol
- PBS
- 0.1-0.25% Trypsin in a balance salt solution
- 2-mercaptoethanol
- Nuclease-free pipette tips
- Nuclease-free 1.5 mL microcentrifuge tubes

Before Starting:

- Prepare RNA Wash Buffer II, DNA Wash Buffer, HBC Buffer, and GTC Lysis Buffer according to the instructions in the Preparing Reagents section on Page 4

1. Determine the proper amount of starting material.

   Note: It is critical to use the correct amount of cultured cells in order to obtain optimal yield and purity with the HiBind® RNA Mini Column. The maximum amount of cells that can be processed with the DNA/RNA Protocol is dependent on the cell line and its RNA content. The maximum binding capacity of the HiBind® RNA Mini Column is 100 µg. The maximum binding capacity of the HiBind® DNA Mini Column is 20 µg. The maximum number of cells that GTC Lysis Buffer can efficiently lyse is 1 x 10^7. Use the following table as a guideline to select the correct amount of starting material. If no information regarding your starting material is available, begin with 1 x 10^6 cells. Based on yield and quality obtained from 1 x 10^6 cells, the starting amount can be adjusted for the next purification.

Source	Number of Cells	RNA Yield (µg)
IC21	1 x 10^6	12
HeLa	1 x 10^6	15
293HEK	1 x 10^6	10
HIN3T3	1 x 10^6	15

2. Harvest cells using one of the following methods. Do not use more than 1 x 10^7 cells.

   - For cells grown in suspension:

     1. Determine the number of cells.
     2. Centrifuge at 500 x *g* for 5 minutes.
     3. Aspirate and discard the supernatant.
     4. Proceed to Step 3 on Page 10.

   - For cells grown in a monolayer:

     Note: These cells can either be lysed directly in the cell culture dish or trypsinized and collected as a cell pellet prior to lysis. Cells grown in cell culture flasks should always be trypsinized.

     - For direct cell lysis:

       1. Determine the number of cells.
       2. Aspirate and discard the cell culture medium.
       3. Immediately proceed to Step 3 on Page 10.

          Note: Incomplete removal of the cell-culture medium will inhibit lysis and dilute the lysate. This will affect the RNA binding conditions of the HiBind® RNA Mini Column and may reduce RNA yield.

     - To trypsinize and collect cells:

       1. Determine the number of cells.
       2. Aspirate and discard the cell-culture medium and wash the cells with PBS.

          Note: Incomplete removal of the cell-culture medium will inhibit trypsin. Multiple washes may be necessary for cells that are difficult to detach.

       3. Add 0.1-0.25% Trypsin in a balanced salt solution.

4. Incubate for 3-5 minutes to allow cells to detach. Check cells for detachment before proceeding to the next step.

5. Add an equal volume of cell-culture medium containing serum to inactivate the trypsin.

6. Transfer cells to an RNase-free glass or polypropylene centrifuge tube (not supplied).

7. Centrifuge at 500 x *g* for 5 minutes.

8. Aspirate the supernatant.

9. Proceed to Step 3 below.

   Note: Incomplete removal of the cell-culture medium will inhibit lysis and dilute the lysate. This will affect the conditions for binding of RNA to the HiBind® RNA Mini Column and may reduce RNA yield.

3. Disrupt cells (do not use more than 1 x 10^7 cells) with GTC Lysis Buffer. Vortex or pipet up and down to mix thoroughly.

   Note: For pelleted cells, loosen the cell pellet thoroughly by flicking the tube before adding the appropriate amount of GTC Lysis Buffer based on the table below. To directly lyse the cells in the culture dish, add the appropriate amount of GTC Lysis Buffer directly to the dish. Collect the cell lysate with a rubber policemen and transfer the cell lysate into a 1.5 mL microcentrifuge tube.

   Note: Add 20 µL 2-mercaptoethanol per 1 mL GTC Lysis Buffer before use. Please see Page 4 for instructions.

Number of Cells	Amount of GTC Lysis Buffer (µL)
< 5 x 10^6	350 µL
5 x 10^6 - 1 x 10^7	700 µL

4. Homogenize the cells accordingly to one of the following methods:

   - Syringe and Needle: Shear high molecular-weight DNA by passing the lysate through a narrow needle (19-21 gauge) 5-10 times.

   - Homogenizer Mini Column (HCR003): Load the lysate into a Homogenizer Mini Column pre-inserted into a 2 mL Collection Tube. Centrifuge at maximum speed for two minutes to collect the homogenized lysate.

   Note: Incomplete homogenization of the sample may cause the column to clog resulting in decreased yields.

RNA Isolation Protocol

5. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

6. Transfer the homogenized lysate to the HiBind® DNA Mini Column.

7. Centrifuge at 13,000 x *g* for 1 minute.

   Note: Make sure that all the lysate has passed through the column after the centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane.

8. Remove and save the HiBind® DNA Mini Column for DNA isolation (Page 13, Steps 27-38).

9. Use the filtrate for RNA isolation in the next step.

10. Add 1 volume 70% ethanol. Vortex to mix thoroughly. Do not centrifuge.

11. Insert a HiBind® RNA Mini Column into a 2 mL Collection Tube.

12. Transfer 700 µL sample (including any precipitate that may have formed) to the HiBind® RNA Mini Column.

13. Centrifuge at 10,000 x *g* for 1 minute.

14. Discard the filtrate and reuse the Collection Tube.

15. Repeat Steps 12-14 until all of the sample has been transferred to the column.

16. Add 500 µL RNA Wash Buffer I to the HiBind® RNA Mini Column.

17. Centrifuge at 10,000 x *g* for 1 minute.

18. Discard the filtrate and reuse the Collection Tube.

19. Add 500 µL RNA Wash Buffer II to the HiBind® RNA Mini Column.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol before use. Please see Page 4 for instructions.

20. Centrifuge at 10,000 x *g* for 1 minute.

21. Discard the filtrate and the Collection Tube.

22. Repeat Steps 19-21 for a second RNA Wash Buffer II wash step.

23. Centrifuge at maximum speed for 2 minutes to completely dry the HiBind® RNA Mini Column.

    Note: It is important to dry the HiBind® RNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

24. Transfer the HiBind® RNA Mini Column to a clean 1.5 mL microcentrifuge tube (not provided).

25. Add 40-70 µL Nuclease-free Water.

    Note: Make sure to add water directly onto the HiBind® RNA Mini Column matrix.

26. Centrifuge at 10,000 x *g* for 1 minute and store eluted RNA at -70°C.

    Note: Any combination of the following steps can be used to help increase RNA yield.

    - Heat the Nuclease-free Water to 70°C before adding to the column.
    - Let the column sit for 5 minutes at room temperature before centrifugation.
    - Increase the elution volume.
    - Repeat the elution step with fresh Nuclease-free Water (this may increase the yield, but decrease the concentration).
    - Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

DNA Isolation Protocol

27. Insert the HiBind® DNA Mini Column from Step 8 into a 2 mL Collection Tube.

28. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with 100% isopropanol before use. Please see Page 4 for instructions.

29. Centrifuge at 10,000 x *g* for 1 minute.

30. Discard the filtrate and reuse collection tube.

31. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

32. Centrifuge at 10,000 x *g* for 1 minute.

33. Discard the filtrate and reuse the collection tube.

34. Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at 10,000 x *g* to dry the column matrix.

    Note: It is important to dry the HiBind® DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

35. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

36. Add 50-100 µL Elution Buffer directly to the center of the column membrane.

37. Centrifuge at 10,000 x *g* for 1 minute.

    Note: This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

38. Store DNA at -20°C.

E.Z.N.A.® DNA/RNA Kit - Animal Tissue Protocol

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 14,000 x *g*
- 70% ethanol
- 100% ethanol
- 100% isopropanol
- 2-mercaptoethanol
- Nuclease-free pipette tips
- Nuclease-free 1.5 mL microcentrifuge tubes

Before Starting:

- Prepare RNA Wash Buffer II, DNA Wash Buffer, HBC Buffer, and GTC Lysis Buffer according to the instructions in the Preparing Reagents section on Page 4

1. Determine the proper amount of starting material.

   Note: It is critical to use the correct amount of cultured cells in order to obtain optimal yield and purity with the HiBind® RNA Mini Column. The maximum amount of cells that can be processed with the DNA/RNA Protocol is dependent on the tissue type and its RNA content. The maximum binding capacity of the HiBind® RNA Mini Column is 100 µg. The maximum binding capacity of the HiBind® DNA Mini Column is 20 µg. The maximum amount of tissue that GTC Lysis Buffer can efficiently lyse is 30 mg. Use the following table as a guideline to select the correct amount of starting material. If no information regarding your starting material is available, begin with 10 mg. Based on yield and quality obtained from 10 mg, the starting amount can be adjusted for the next purification.

Average Yield of Total Cellular RNA from Mouse Tissue

Source	Amount of Tissue (mg)	RNA Yield (µg)
Brain	10	10
Kidney	10	30
Liver	10	45
Heart	10	5
Spleen	10	33
Lung	10	12
Pancreas	10	40
Thymus	10	20

2. Homogenize and disrupt the tissue according to one of the methods described on Pages 6-7.

   Note: Add 20 µl 2-mercaptoethanol per 1 mL GTC Lysis Buffer before use. Please see Page 4 for instructions.

   Note: Incomplete homogenization of the sample may cause the column to clog resulting in decreased yield. It is recommended to homogenize the tissue samples with a rotor-stator homogenizer as this method normally produces better yields.

   Amount of GTC Lysis Buffer per Tissue Sample

Amount of Tissue	Amount of GTC Lysis Buffer (µL)
≤ 15 mg	350 µL
20-30 mg	700 µL

3. Centrifuge at 13,000 x *g* for 5 minutes.

4. Transfer the cleared supernatant to a clean 1.5 mL microcentrifuge tube (not supplied).

   Note: In some preparations, a fatty upper layer will form after centrifugation. Transfer of any of the fatty upper layer may reduce RNA yield or clog the column.

RNA Isolation Protocol

5. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

6. Transfer the homogenized lysate to the HiBind® DNA Mini Column.

7. Centrifuge at ≥13,000 x *g* for 1 minute.

   Note: Make sure that all the lysate has passed through the column after the centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane.

8. Remove and save the HiBind® DNA Mini Column for DNA isolation (Page 18, Steps 27-38).

9. Use the filtrate for RNA isolation in the next step.

10. Add 1 volume 70% ethanol. Vortex to mix thoroughly. Do not centrifuge.

11. Insert a HiBind® RNA Mini Column into a 2 mL Collection Tube.

12. Transfer 700 µL sample (including any precipitate that may have formed) to the HiBind® RNA Mini Column.

13. Centrifuge at 10,000 x *g* for 1 minute.

14. Discard the filtrate and reuse the Collection Tube.

15. Repeat Steps 12-14 until all of the sample has been transferred to the column.

16. Add 500 µL RNA Wash Buffer I to the HiBind® RNA Mini Column.

17. Centrifuge at 10,000 x *g* for 1 minute.

18. Discard the filtrate and reuse the Collection Tube.

19. Add 500 µL RNA Wash Buffer II to the HiBind® RNA Mini Column.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol before use. Please see Page 4 for instructions.

20. Centrifuge at 10,000 x *g* for 1 minute.

21. Discard the filtrate and reuse the Collection Tube.

22. Repeat Steps 19-21 for a second RNA Wash Buffer II wash step.

23. Centrifuge at maximum speed for 2 minutes to completely dry the HiBind® RNA Mini Column.

    Note: It is important to dry the HiBind® RNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

24. Transfer the HiBind® RNA Mini Column to a clean 1.5 mL microcentrifuge tube (not provided).

25. Add 40-70 µL Nuclease-free Water.

    Note: Make sure to add water directly onto the HiBind® RNA Mini Column matrix.

26. Centrifuge at 10,000 x *g* for 1 minute and store eluted RNA at -70°C.

    Note: Any combination of the following steps can be used to help increase RNA yield.

    - Heat the Nuclease-free Water to 70°C before adding to the column.
    - Let the column sit for 5 minutes at room temperature before centrifugation.
    - Increase the elution volume.
    - Repeat the elution step with fresh Nuclease-free Water (this may increase the yield, but decrease the concentration).
    - Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

DNA Isolation Protocol

27. Insert the HiBind® DNA Mini Column from Step 8 into a 2 mL Collection Tube.

28. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with 100% isopropanol before use. Please see Page 4 for instructions.

29. Centrifuge at 10,000 x *g* for 1 minute.

30. Discard the filtrate and reuse collection tube.

31. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

32. Centrifuge at 10,000 x *g* for 1 minute.

33. Discard the filtrate and reuse the collection tube.

34. Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at 10,000 x *g* to dry the column matrix.

    Note: It is important to dry the HiBind® DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

35. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

36. Add 50-100 µL Elution Buffer directly to the center of the column membrane.

37. Centrifuge at 10,000 x *g* for 1 minute.

    Note: This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

38. Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
Little or no RNA eluted	RNA remains on the column	• Repeat elution. • Heat Nuclease-free Water to 70°C prior to elution. • Incubate column for 10 minutes with water prior to centrifugation.
	Column is overloaded	Reduce the quantity of the starting material.
Problem	Cause	Solution
Degraded RNA	Source	• Freeze starting material quickly in liquid nitrogen. • Do not store tissue cultured cells prior to extraction unless they are lysed first. • Follow protocol closely and work quickly.
	RNase contamination	• Ensure not to introduce RNase during the procedure. • Check buffers for RNase contamination.
Problem		Solution
Clogged column	Incomplete homogenization	• Completely homogenize sample. • Increase centrifugation time. • Reduce the amount of the starting material.
Problem in downstream applications	Salt carryover during elution	• Ensure RNA Wash Buffer II has been diluted with 4 volumes of 100% ethanol as indicated on bottle. • RNA Wash Buffer II must be stored and used at room temperature. • Repeat wash with RNA Wash Buffer II.
DNA contamination	HiBind® DNA Mini Column is overloaded	• Reduce the amount of starting material. • Digest with RNase-free DNase and inactivate at 75°C for 5 minutes.
Low Abs ratios	RNA diluted in acidic buffer or water	• Nuclease-free water is slightly acidic and can lower A_{260}/A_{280} ratios. Use TE buffer to dilute RNA prior to spectrophotometric analysis.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
Elution Buffer (100 mL)	PDR048
DNA Wash Buffer (100 mL)	PS010
RNA Wash Buffer II (25 mL)	PR031
Nuclease-free Water (1000 mL)	PD092
2 mL Collection Tubes (500/pk)	SSI-1370-00-01
2 mL Collection Tubes (500/pk, 50 pk/cs)	SSI-1370-00
1.5 mL DNase/RNase-free Microcentrifuge Tubes (500/pk, 10 pk/cs)	SS1-1210-00
Omega Homogenizer Columns (200 preps)	HCR003

Notes:

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Notes:

For more purification solutions, visit **www.omegabiotek.com**

AVAILABLE FORMATS


Spin Columns

96-Well Silica Plates


Mag Beads

SAMPLE TYPES


Blood / Plasma

Plasmid


Cultured Cells


Plant & Soil


NGS Clean Up


Tissue


FFPE


Fecal Matter

omega BIO-TEK

innovations in nucleic acid isolation

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