Genomic DNA Isolation/ Total RNA Purification NucleoBond®

User manual

NucleoBond® RNA/DNA kits

NucleoBond® CB kits

NucleoBond® AXG columns

May 2006/Rev. 02

MACHEREY-NAGEL

Table of contents

1 Kit contents

	NucleoBond® RNA/DNA 80	NucleoBond® RNA/DNA 400
	25 preps	**10 preps**
Cat. No.	**740650**	**740651**
Buffer W1	20 ml	35 ml
Buffer W3	10 ml	10 ml
Buffer W4	15 ml	25 ml
Buffer W5	35 ml	80 ml
Buffer W6	15 ml	25 ml
Buffer R0	2 x 125 ml	4 x 100 ml
Buffer R1	2 x 120 ml	2 x 100 ml
Buffer R2	2 x 120 ml	2 x 80 ml
Buffer R3	2 x 100 ml	125 ml
Buffer R4	75 ml	75 ml
Buffer N5	90 ml	70 ml
Urea	36.25 g	36.25 g
NucleoBond® AXR 80 columns	25	-
NucleoBond® AXR 400 columns	-	10
Plastic washer	10	5
Protocol	1	1

1 Kit contents *continued*

	NucleoBond® CB 100	NucleoBond® CB 500
	20 preps	**10 preps**
Cat. No.	**740508**	**740509**
Buffer G1	2 x 63 ml	2 x 107 ml
Buffer G2	110 ml	125 ml
Buffer N2	2 x 70 ml	2 x 80 ml
Buffer N3	2 x 125 ml	2 x 125 ml
Buffer N5	2 x 100 ml	2 x 90 ml
Proteinase K	40 mg (dissolve in 2 ml of Proteinase Buffer)	40 mg (dissolve in 2 ml of Proteinase Buffer)
Proteinase Buffer	3.6 ml	3.6 ml
NucleoBond® AXG 100 columns	20	-
NucleoBond® AXG 500 columns	-	10
Plastic washer	10	5
Protocol	1	1

1 Kit contents *continued*

	NucleoBond® Buffer Set III	NucleoBond® Buffer Set IV
Application	**genomic DNA from bacteria**	**genomic DNA from tissue**
Cat. No.	**740603**	**740604**
Buffer S1	-	-
Buffer S2	-	-
Buffer S3	-	-
Buffer G2	-	2 x 125 ml
Buffer G3	2 x 70 ml	-
Buffer G4	40 ml	-
Buffer N2	2 x 125 ml	3 x 100 ml
Buffer N3	3 x 100 ml	3 x 100 ml
Buffer N5	2 x 100 ml	2 x 100 ml
RNase A	2 x 15 mg	2 x 25 mg
Proteinase K	2 x 50 mg (dissolve in 2.5 ml of Proteinase Buffer each)	2 x 50 mg (dissolve in 2.5 ml of Proteinase Buffer each)
Proteinase Buffer	8 ml	8 ml
Protocol	1	1

1 Kit contents *continued*

	NucleoBond® AXG 20	NucleoBond® AXG 100	NucleoBond® AXG 500
Cat. No.	**740544**	**740545**	**740546**
NucleoBond® AXG 20 columns	20	-	-
NucleoBond® AXG 100 columns	-	20	-
NucleoBond® AXG 500 columns	-	-	10
Plastic washer	10	10	5
Protocol	1	1	1

2 Product description

2.1 Properties

NucleoBond® A X is a silica-based, outstanding anion exchanger developed and manufactured by MACHEREY-NAGEL for routine separation of different classes of nucleic acids; it is covered by European patent EP 0496822.
The outstanding purity of this macroporous silica is guaranteed by production in our facilities and is an important prerequisite for the superior biological activity of the purified nucleic acids. It enables high mass recoveries by minimizing nonspecific adsorption effects. The resolving power of **NucleoBond® AX** is illustrated in figure 1.

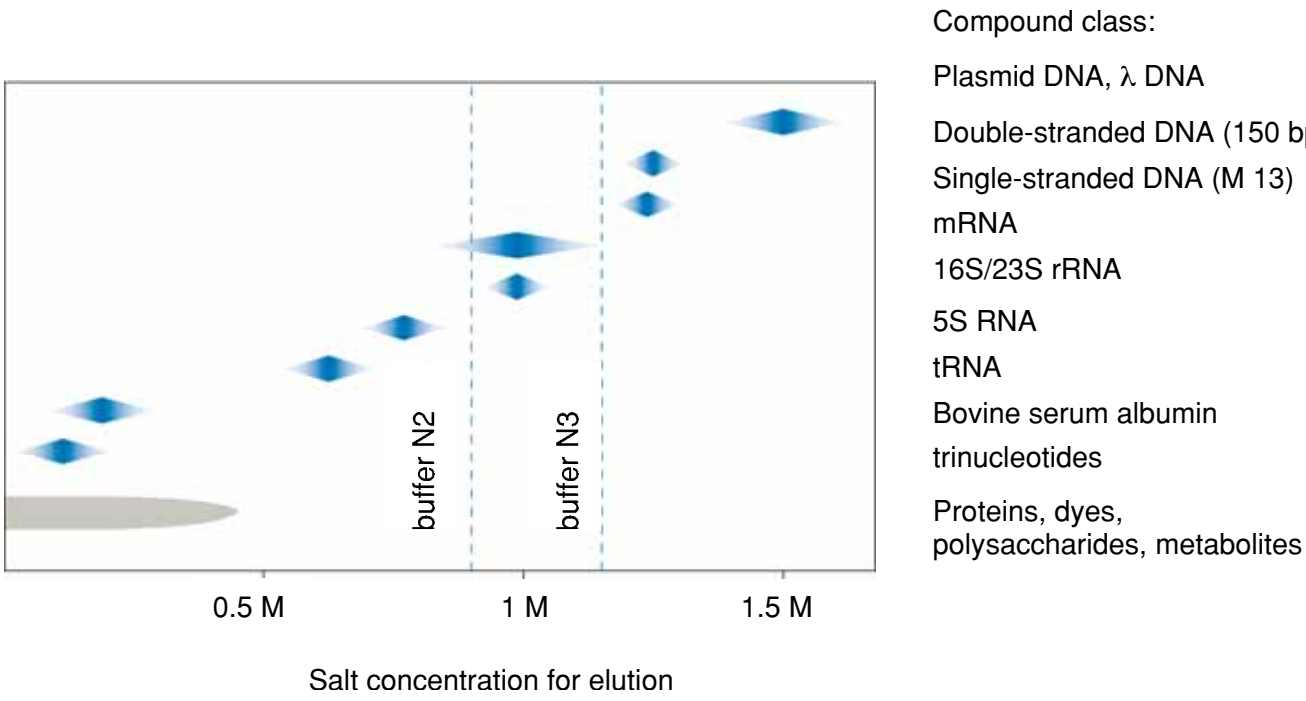

Figure 1: Elution profiles for distinct nucleic acid species using NucleoBond® AX columns. Nucleic acids can be eluted over a range of 0.5 M KCl to 1.5 M KCl; profiles for each nucleic acid are sharp and virtually non-overlapping.

The extraordinary high charge density on its hydrophilic, macroporous surface results in a salt concentration range for binding and elution of nucleic acids which is much larger than on conventional anion exchangers.

These are the reasons for the superior resolving power of **NucleoBond® AX** for easy separation of nucleic acids from one another and from proteins, carbohydrates, metabolites etc. with an efficiency not achieved by usual ion exchangers or by gel filtration.

2.2 NucleoBond® columns

The NucleoBond® ion exchange silica is secured between inert filter elements in polypropylene columns resulting in an inert and efficient separation tool. NucleoBond® AX columns come in five different sizes covering the whole purification range from nanogram amounts to 10 mg DNA (see table 1).

No expensive instrumentation such as ultracentrifuges or HPLC equipment is needed to get a result at least as good as by CsCl gradients.

All nucleic acids purified show absorption ratios of $A_{260/280}$ as high as 1.8 – 2.0. Low amounts of impurities, which often act as strong inhibitors for enzymatic processing, sequencing, transfections, transcriptions etc., are removed completely.

Table 1: Binding capacities of the NucleoBond® columns

NucleoBond® type	Capacities
AXG 20	20 µg genomic DNA
AXG 100	100 µg genomic DNA
AXG 500	500 µg genomic DNA
AXR 80	80 µg total RNA
AXR 400	400 µg total RNA

NucleoBond® columns are universal tools especially for the purification of nucleic acids. They can be used for the fast isolation of

– genomic DNA from blood, cell cultures, bacteria, tissue, and yeast
– total RNA from eukaryotic cells, bacteria, tissue and yeast
– rRNAs / mRNA / group of tRNAs
– large RNA fragments
– RNA from reaction mixtures

Fast operation procedures can be used for all these purifications on **NucleoBond®** columns. The usual time scale is minutes, not hours. Using the appropriate working procedure, oligos, RNA or different types of DNA are purified on **NucleoBond®** with high recovery. Small or large amounts of plasmids and RNAs are eluted from this purification tool by more than 90%.

Since the over-all yield for the purification of cellular nucleic acids depends on variables like cell type, culture conditions, efficiency of cell lysis etc. these factors may change the result and should therefore be taken into consideration. All **NucleoBond®** types are resistant to organic solvents like alcohol, chloroform and phenol. They are free of RNase and DNase.

Often there is not enough time to make up the whole set of buffers and other solutions needed for purifying a certain nucleic acid. In these cases NucleoBond® KITs should be used. Kits are available for:

Genomic DNA from blood and cell cultures	**NucleoBond® CB Kit**
Total RNA / DNA from eukaryotic cells, bacteria, tissue and yeast	**NucleoBond® RNA/DNA Kit**

They are the fastest, most reliable and easiest way to get a high quality nucleic acid in a high over-all yield.

NucleoBond® Kits are ready-to-use separation systems containing:

- columns
- appropriate buffers, solutions, and reagents
- complete protocols

2.3 NucleoBond® buffers

Table 2: NucleoBond® buffer compositions

Buffer	Buffering compounds	Storage	Application
G1	320 mM saccharose, 5 mM $MgCl_2$, 10 mM Tris/HCl, 1% Triton X-100, pH 7.5	4°C	For genomic DNA from blood, cell cultures, bacteria and tissue
G2	800 mM GuHCl, 30 mM EDTA, 30 mM Tris/HCl, 5% Tween 20, 0.5% Triton X-100, pH 8.0	4°C	
G3	50 mM EDTA, 50 mM Tris/HCl, 0.5% Tween 20, 0.5% Triton X-100, pH 8.0	4°C	
G4	3 M GuHCl, 20% Tween 20, pH 5.5	4°C	
R0	100 mM Tris/acetate,15% ethanol, pH 6.3	RT	For total RNA from eukaryotic calls, bacteria, tissue and yeast
R1	100 mM Tris/acetate,15% ethanol, 400 mM KCl, pH 6.3	RT	
R2	100 mM Tris/acetate,15% ethanol, 900 mM KCl, pH 6.3	RT	
R3	100 mM Tris/acetate,15% ethanol, 1150 mM KCl, pH 6.3	RT	
R4	100 mM Tris/acetate,15% ethanol, 1150 mM KCl, 6 M urea, pH 6.3	RT	
W1	50 mM Tris/acetate, 4 M GuSCN, pH 7.8	4°C	
W3	20% Triton X-100	RT	
W4	3 M sodium acetate, pH 6.5	RT	For total RNA from eukaryotic calls, bacteria, tissue and yeast
W5	10 mM Tris/acetate, 1 mM EDTA pH 7.8	RT	
W6	200 mM Tris/acetate, 1.5 M KCl, pH 6.3	RT	
N2	100 mM Tris/H_3PO_4, 15% ethanol, 900 mM KCl, pH 6.3, 0.15% Triton X-100	RT	Column buffers
N3	100 mM Tris/H_3PO_4, 15% ethanol, 1150 mM KCl, pH 6.3	RT	
N5	100 mM Tris/H_3PO_4, 15% ethanol, 1000 mM KCl, pH 8.5	RT	

All separations are made in aqueous buffers with ethanol as modifier. Low salt concentrations of sample are needed for the binding of nucleic acids to this macroporous anion exchanger. This salt concentration is increased stepwise in the following washing and elution steps.

The salt concentration for elution of a given nucleic acid depends on the pH value of the eluent. This is why the pH of the buffers should be carefully controlled. A deviation of more than 0.1 pH units from the given values will result in changed salt concentration for the elution of each nucleic acid, as shown in figure 2.

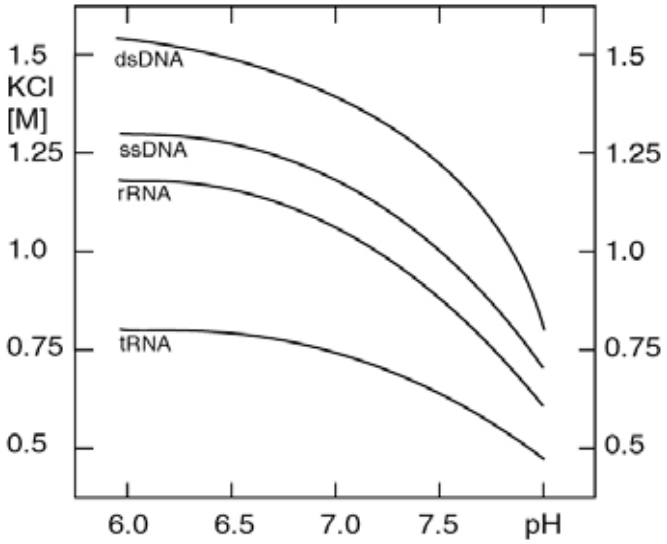

Figure 2:
Elution of nucleic acids depending on the pH of the buffer; 100 mM Tris/H_3PO_4, 15 % ethanol

2.4 General procedure

NucleoBond® AX can be used in the pH range 2.5 – 8.5 for about 3 hours without any change in its chromatographic properties. After that time nucleic acids will elute at increasingly lower salt concentrations. The columns may be used at salt concentrations up to 2 M for elution. They are not damaged in the presence of denaturing agents like formamide or urea, nonionic detergents such as Triton X-100, and most organic solvents.

NucleoBond® AX columns are easy to handle. The easiest and most effective technique of passing buffer solutions through these columns is the gravity flow. Solutions are pipetted or just poured into the columns. All types of NucleoBond® AX columns may be used in this mode. They do not run dry.

The supplied PE tubing (only for AX 2000) should be connected to the column before use, to reach the optimum flow rate. All types of NucleoBond® AX columns may be stationed in test tubes or fixed by the use of clamps (see figure 3).

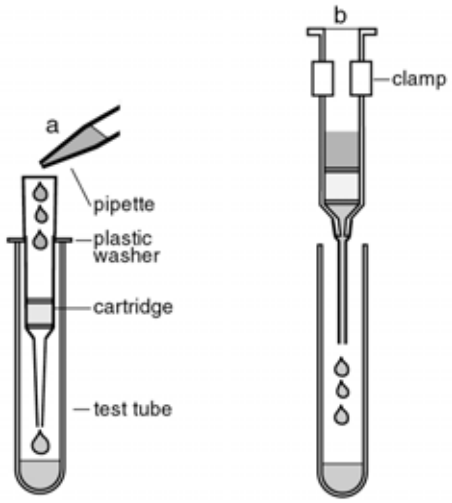

Figure 3

In all cases a simple and time-saving basic procedure is used:

1. **Equilibration of NucleoBond® AX with the binding buffer**
2. **Adsorption of the nucleic acids from the solution (cleared lysate, enzymatic reaction mixture, or extract from gels)**
3. **Washing step (removes all contaminants)**
4. **Elution of the desired nucleic acid**

Equilibration step
Fill the appropriate volume of equilibration buffer into the column. The buffer solution passes through the column by gravity flow. The flow-through stops, when the surface of the buffer solution reaches the upper filter element. The remaining buffer assures an immediate start of the flow in all following steps. It should not be pushed out.

Adsorption of the sample
The clear sample should be loaded onto the column by gravity flow.

Washing step
It removes all contaminating substances. Washing is performed, in principle, like the equilibration step.

Elution of nucleic acids
Elution is carried out into a new tube with the volume of elution buffer indicated in the corresponding protocol. Since the column has a certain dead volume, the first part of the flow-through may be discarded. By this treatment the nucleic acid is collected in the lowest amount of elution buffer. The nucleic acid is precipitated by addition of 0.7 – 0.8 volumes of isopropanol at room temperature. Don't let the DNA solution drop into a vial with isopropanol, because this leads to a spontaneous coprecipitation of the salt. If only small amounts of nucleic acids are present, the pH should be adjusted to 5.2 before starting the precipitation step.

Storage conditions
Store the kits at room temperature.
After preparation of buffers G2, G3 (with RNase A), and buffer G1 (with saccharose) these buffers should be stored at 4 °C.
Dissolved proteinase K solution should be stored at 4°C (see individual protocols).

2.5 Quality control

Nucleic acids of high purity
Because of the high selectivity of **NucleoBond® AX**, very pure nucleic acids are obtained with this fast and efficient separation tool. No expensive instrumentation such as ultracentrifuges or HPLC equipment is needed to get a result at least as good as by CsCl gradients. It takes only minutes to isolate DNA free from any kind of RNA, proteins, metabolites, dyes or carbohydrates. The same is true for RNA. All nucleic acids purified, show absorption ratios of A260/A280 as high as 1.8 – 2.0.
Low amounts of impurities, which often act as strong inhibitors for enzymatic processing, sequencing, transfections, transcriptions etc., are removed completely.

3. NucleoBond® Applications

3.1 Summary

Table 3: Buffer sequence for different applications of NucleoBond®

Applications	Equilibration of the column	Adsorption of the sample [KCl]	Washing step	Elution of the nucleic acid
1. Purification of genomic DNA	buffer N2	850 mM	buffer N3	buffer N5
2. Purification of total RNA	buffer R1	350 mM	buffer R1, R2	buffer R3, R4
3. Removal of linker DNA from DNA fragments (>150 base pairs) in cloning experiments	buffer N1 (N2)*	650 mM	buffer N3	buffer N5

* for DNA fragments larger than 100 bp

3.2 Isolation of total RNA / genomic DNA from eukaryotic cells, bacteria, tissue, and yeast

NucleoBond® RNA/DNA 80 and 400 kits are designed for the isolation and purification of up to 80 or 400 µg total RNA and genomic DNA from eukaryotic cells, bacteria, tissue and yeast.

The NucleoBond® RNA/DNA kits include columns, all required buffer solutions and protocols. Lysozyme, lyticase and DNase as well as sorbitol buffer are not included in the kit.

Due to the superior resolving power of NucleoBond® RNA/DNA, contaminations are washed away while RNA and genomic DNA can be eluted widely separate with washing/elution buffers R1, R2, R3, R4 and N5 of increasing stringency. Due to the RNA species of interest different washing/elution buffers are recommended as indicated in the protocol:

- a gentle elution of RNA with buffer R3 of less ionic strength causes reduced yield but increased purity, and nearly avoids DNA contamination;
- buffer R4 containing a higher ionic strength than R3 causes higher yield;
- generally, the elution behaviour of RNA depends on its size and source. To get preliminary information elute with buffer R3, afterwards with buffer R4, and than decide which buffer system has to be used.

Table 4: Elution conditions for different RNA species

Compound	KCl salt concentration for elution (buffer R: 100 mM Tris/acetate, 15% ethanol, pH 6.3)
tRNA	0.45 – 0.65 M
rRNA	0.95 – 1.10 M
mRNA	0.70 – 1.15 M
5S rRNA	0.65 – 0.85 M
total RNA	0.45 – 1.15 M

Due to the different cell content and proportions of cellular components the following protocols include several options which may be tested and compared with different amounts of cells/tissue. Do not overload the column because this will result in decreased yield and purity of RNA preparations. If you are in doubt, use first a small amount of sample in order to find out the RNA content. Afterwards, use the appropriate amount of sample according to the limited lysing capacity of the lysis buffer and according to the capacity of the column as indicated in the protocol.

During the homogenization process the isolated genomic DNA can be sheared into small fragments and may partially appear in the RNA fraction. If a complete removal of genomic DNA is required, a DNase treatment after the precipitation of RNA or e.g. a subsequent LiCl precipitation of the isolated nucleic acids is recommended.

3.2.1 Isolation of total RNA and genomic DNA from bacteria, yeast, and small amounts of eukaryotic cells

Step of the procedure	Special conditions for NucleoBond® columns	
	AXR 80	AXR 400
maximum binding capacity	80 µg	400 µg
recommended number of bacterial cells	0.5×10^8	2×10^9
recommended number of eukaryotic cells	$10^5 – 10^6$	$1 – 5 \times 10^6$
Cell lysis and separation of proteins		
1A. For the isolation of **total RNA from bacteria:**		
An enzymatic treatment before starting the isolation is recommended. For this purpose add e.g. lysozyme (250 – 1000 µg/ml TE, pH 8.0) to the bacterial cell pellet, resuspend it and incubate for 10 min at room temperature. Depending on the bacterial strain other appropriate enzymes are also compatible with this method.	100 µl	400 µl
Afterwards, add buffer W1 and mix carefully.	0.4 ml	1.6 ml
1B. For the isolation of **total RNA from yeast:**		
Harvest the indicated volume of culture (YPD medium) by centrifugation for 10 min at 5.000xg. Resuspend the cell pellet in sorbitol buffer (1 M sorbitol, 100 mM EDTA, 14 mM ß-mercaptoethanol, pH 7.4, not included in our NucleoBond® RNA/DNA kits) containing lyticase or zymolase (50 – 100 U/ml), and incubate for 30min at 30°C. Centrifuge for 10 min at 1.000xg to pellet the spheroblasts. After removal of the supernatant add buffer W1 to the cell pellet and homogenize the lysis mixture by pipetting and vortexing.	3 – 5 ml 1 ml 0.5 ml	5 – 20 ml 4 ml 2 ml
1C. For the isolation of total RNA **from eukaryotic cells:** Add buffer W1 to the cells. Homogenize the lysis mixture by vortexing or the usage of a glas-teflon homogenizer.	0.5 ml	2 ml
2. Add β-mercaptoethanol to the solution and homogenize by vortexing. In order to reduce the viscosity pass the lysate 3 times through a sterile plastic syringe fitted with a 20 gauge needle.	0.5 µl	2 µl
3. Add buffer W3. Mix the sample and incubate for 5 min at 4°C.	50 µl	200 µl
Add buffer W4. The sample is mixed carefully and incubate for 5 min at room temperature. Centrifuge the mixture at 10,000 x g for 20 min at 4°C in order to separate cellular debris and proteins.	500 µl	2 ml
4. Add buffer R0 to the supernatant and mix carefully.	10 ml	36 ml

Step of the procedure	Special conditions for NucleoBond® columns	
	AXR 80	AXR 400
5. If necessary, centrifuge the solution (10 min, 12,000 x g, 4°C) in order to remove insoluble particles and to avoid clogging of the column, and collect the clear supernatant.	optional	optional
Equilibration		
6. Equilibrate a NucleoBond® AXR column with buffer R1.	1 ml	3 ml
Adsorption		
7. Transfer the clear supernatant to the column. Collect the flow-through containing genomic DNA.		
Wash		
8. Wash the NucleoBond® AXR column. Use buffers given below for the purification of certain RNA species: **Buffer R1** for **tRNA** or **total RNA** or **Buffer R1/2 (1:1)** for **rRNA/mRNA** or **Buffer R2** for **rRNA** or **viral RNA** (stringent wash)	6 ml	12 ml
Elution		
9. Elute the RNA with Buffer R4 (prewarming to 50°C improves the yield of total RNA but may increase DNA contamination). Alternatively, first buffer R3 may be used for elution.	3 ml	6 ml
Optional subsequent isolation of genomic DNA:		
a. Apply the flow-through (step 7) to the column and wash the column with buffer R3 to remove residual RNA.	3 ml	6 ml
b. Elute DNA with buffer N5 prewarmed to 50°C.	3 ml	6 ml
Precipitation		
10. Add isopropanol to the RNA (DNA) eluate, mix, incubate for 15 min on ice, and centrifuge for 25 min at 10,000 x g and 4°C.	2.5 ml	5 ml
11. Wash the RNA (DNA) pellet with 85% ethanol, dry the pellet for 5 – 10 min, and dissolve it in an appropriate buffer for further applications.	1 ml	1 ml
If total removal of DNA (or RNA) is necessary for subsequent reactions an additional enzymatic treatment with DNase (or RNase) is recommended. After this treatment the enzymes can be inhibited by appropriate buffers or heat inactivation (DNase). Alternatively, a further purification step with NucleoBond® or NucleoSpin® kits is recommended.		

3.2.2 Isolation of total RNA and genomic DNA from eukaryotic cells and tissue

Step of the procedure		Special conditions for NucleoBond® columns	
		AXR 80	AXR 400
	maximum binding capacity	80 µg	400 µg
	recommended number of cells	1 – 5 x 10^6	5 x 10^6–2 x 10^7
	recommended amount of tissue	20 mg	100 mg
Cell lysis and separation of proteins			
1.	Add buffer W1 to the cells or tissue.	0.5 ml	2 ml
2.	Add ß-mercaptoethanol (not included in our NucleoBond® RNA/DNA kits) to the solution and homogenize 3 – 4 times for each 20 seconds using a commercial homogenizer (e.g. Polytron, Dounce). Alternatively, other homogenization tools like mortar and pestle in the presence of liquid nitrogen may be used. In order to reduce the viscosity pass the lysate 3 times through a sterile plastic syringe fitted with a 20 gauge needle.	0.5 µl	2 µl
3.	Add buffer W3. Mix the sample and incubate it for 15 min at 4°C.	50 µl	200 µl
4.	Add buffer W4. Mix the sample carefully and incubate it for 15 min at 4°C.	500 µl	2 ml
5.	Centrifuge the mixture at 10,000 x g for 20 min at 4°C in order to separate cellular debris.		
Precipitation of nucleic acids			
6.	Add isopropanol to the supernatant, mix carefully and incubate for 10 min on ice.	850 µl	3.4 ml
7.	Centrifuge the mixture at 10,000 x g for 20 min at 4°C.	yes	yes
8.	Discard the supernatant and dissolve the RNA pellet in buffer W1 very carefully, if necessary by incubation at 65°C for 1 – 3 min. Avoid heat incubation because RNA may be damaged by coprecipitated substances. This may be the case, if complex matrices have to be processed which are rich in secondary metabolites. If dissolution in buffer W1 is not possible, you may alternatively use buffers W5 and W6 (see section 3.2.3, step 2). *Optional:* If total removal of DNA is necessary for subsequent reactions, an additional enzymatic treatment with DNase is recommended. Dissolve the pellet in DNase-incubation buffer, proceed with an enzymatic digestion as recommended by the supplier, and finally add buffer W1 as described above (step 8).	0.2 ml 50 µl	0.8 ml 100 – 200 µl

Step of the procedure	Special conditions for NucleoBond® columns	
	AXR 80	AXR 400
9. Remove insoluble particles by centrifugation (10 min, 12,000 x g, 4°C), and collect the supernatant.	optional	optional
10. Add buffer R0 to the supernatant and mix.	2 ml	8 ml
Equilibration		
11. Equilibrate a NucleoBond® AXR column with buffer R1.	1 ml	3 ml
Adsorption		
12. Transfer the sample to the column and collect the flow-through containing DNA.		
Wash		
13. Wash the NucleoBond® AXR column. Use buffers given below for the purification of certain RNA species:	6 ml	12 ml
Buffer R1 for **tRNA** or **total RNA**		
or **Buffer R1/2 (1:1)** for **rRNA/mRNA**		
or **Buffer R2** for **rRNA** or **viral RNA** (stringent wash)		
Elution		
14. Elute the RNA with Buffer R4 (prewarming to 50°C improves yield of total RNA but may increase DNA contamination).	3 ml	6 ml
Optional: Isolation of genomic DNA:		
a. Apply the flow-through (step 12) to the column, and wash the column with buffer R3 to remove residual RNA.	3 ml	6 ml
b. Elute DNA with buffer N5 prewarmed to 50°C.	3 ml	6 ml
Precipitation		
15. Add isopropanol to the eluate, mix, incubate on ice for 15 min, and centrifuge for 25 min at 10,000 x g and 4°C.	2.5 ml	5 ml
16. Wash the RNA (DNA) pellet with 85% ethanol, dry the pellet for 5 – 10 min, and dissolve it in an appropriate buffer for further applications.	1 ml	1 ml
If total removal of DNA (or RNA) is necessary for subsequent reactions an additional enzymatic treatment with DNase (or RNase) is recommended. The enzymes can be inhibited by appropriate buffers or heat inactivation (DNase). Alternatively, a further purification step with NucleoBond® or NucleoSpin® kits is recommended.		

3.2.3 RNA clean-up from reaction mixtures

Generally, RNA-containing reaction mixtures are adjusted to binding conditions by adding 1/5 volume of buffer R3 and then bound to the column. Such reaction mixtures are e.g. pre-purified RNA samples, solutions containing radioactive labelled RNA probes, or "run off" transcripts in low salt solutions. Good binding conditions for different RNAs to **NucleoBond® AXR** are pH 6.3 and a final salt concentration of 0.2 M, e.g. KCl.

For RNA-containing samples which require a further cell lysis (e.g. body fluids) and for solid samples (e.g. RNA pellets) the following procedure is recommended:

Step of the procedure	Special conditions for NucleoBond® columns	
	AXR 80	AXR 400
maximum binding capacity	80 µg	400 µg
maximum volumes for fluid samples, reaction mixtures	100 µl	400 µl
Adjustment of binding conditions		
1.a **For RNA-containing fluid samples or reaction mixtures:**		
Use the indicated volumes. If doubled volumes have to be processed use doubled volumes of buffers W1, W3, and R0. Afterwards, add buffer W1 and proceed with step 2.	10 – 100 µl 0.4 ml	40 – 400 µl 1.6 ml
1.b **For solid samples (e.g. RNA pellets):**		
Add buffer W5. Dissolve the pellet very carefully, if necessary, by incubation at 65°C for 1 – 3 min. Afterwards, add buffer W6, mix and proceed with step 4.	1.2 ml 400 µl	7.5 ml 2.5 ml
2. Add buffer W3. The sample is mixed and incubate for 5 min at room temperature.	50 µl	200 µl
3. Add buffer R0 to the supernatant and mix carefully.	5 ml	18 ml
4. If necessary, centrifuge the solution (10 min, 12,000 x g, 4°C) in order to remove insoluble particles and to avoid clogging of the column, and collect the clear supernatant.		
Equilibration		
5. Equilibrate a NucleoBond® AXR column with buffer R1.	1 ml	3 ml
Adsorption		
6. Transfer the clear supernatant to the column.		
Wash		
7. Wash the NucleoBond® AXR column. Use buffers given below for the purification of certain RNA species: **Buffer R1** for **tRNA** or **total RNA** or **Buffer R1/2 (1:1)** for **rRNA/mRNA** or **Buffer R2** for **rRNA** or **viral RNA** (stringent wash)	6 ml	12 ml

Step of the procedure	Special conditions for NucleoBond® columns	
	AXR 80	AXR 400
Elution		
8. Elute the RNA with Buffer R3 prewarmed to 50°C.	3 ml	6 ml
Precipitation		
9. Add isopropanol to the eluate, mix, incubate for 15 min on ice and centrifuge for 25 min at 10,000 x g and 4°C.	2.5 ml	5 ml
10. The RNA pellet is washed with 85% ethanol, dried for 5 – 10 min and dissolved in an appropriate buffer for further applications. If total removal of DNA is necessary for subsequent reactions, an additional enzymatic treatment with DNase is recommended. The enzyme can be inhibited by appropriate buffers or heat inactivation.	1 ml	1 ml

3.3 Isolation of genomic DNA from blood and cell cultures

For the isolation of genomic DNA from up to 20 ml whole blood, up to 1 ml of buffy coat, or up to 1 x 10^8 cultured cells we recommend our NucleoBond® CB kits. These kits include columns, buffer solutions as well as proteinase K. Blood treated either with citrate, heparin, or EDTA can be used.

Before starting the procedure, please read the remarks below:

Buffer G1: Store buffer G1 at 4°C.
Before use add saccharose, which is included in the kit, to buffer G1. After addition of saccharose to G1 the buffer has to be stored at 4°C for a maximum of 3 months.

Buffer G2: After the first use buffer G2 should be stored at 4 °C and has to be equilibrated to room temperature before use.

Proteinase K: Dissolve proteinase K in **Proteinase Buffer** (as indicated on the bottle, see also section 1, Kit contents). The dissolved proteinase K is stable at 4°C for 6 months under these conditions. Divide the solution into small aliquots and store at –20°C if the solution will not be used up during this period. Frozen proteinase K stock solutions are stable up to one year.

Before starting the procedure equilibrate 20 ml **ddH_2O** on ice.

Step of the procedure	Special conditions for NucleoBond® columns		
	AXG 20	AXG 100	AXG 500
maximum yield of genomic DNA	20 µg	100 µg	500 µg
typical amount of cells	5 x 10^6	2 x 10^7	1 x 10^8
volume of blood	0.1 – 1 ml	2 – 5 ml	5 – 20 ml
volume of buffy coat	< 50 µl	< 250 µl	1 ml
Cell disruption			
Cell culture: After washing the cells twice with PBS and centrifugation resuspend the cells in PBS to a final concentration of 10^7 cells/ml.			
1. Add 1 volume of buffer G1 (ice-cold) and 3 volumes ddH_2O (ice-cold) to 1 volume whole blood or cell suspension. Example: For 1 ml cell suspension (~10^7 cells) or 1 ml blood add 1 ml of buffer G1 and 3 ml ddH_2O.	1 vol G1 3 vol ddH_2O	1 vol G1 3 vol ddH_2O	1 vol G1 3 vol ddH_2O
2. Mix the suspension by inverting the tube 6 – 8 times and incubate the mixture for 10 min on ice.			
3. Centrifuge the mixture at 4°C (important) for 15 min at 1,300 – 1,500 x g (around 3,500 rpm). Discard the supernatant. A small red pellet is visible.			

Step of the procedure	Special conditions for NucleoBond® columns		
	AXG 20	AXG 100	AXG 500
4. Add buffer G1 (ice-cold) and ddH_2O (ice-cold) and resuspend the pellet by vortexing (~ 5 – 10 sec). Centrifuge the mixture at 4°C (important) for 15 min at 1,300 – 1,500 x g (around 3,500 rpm). Discard the supernatant. The pellet should be almost white. Small red spots on the pellet are not critical for the procedure. If the whole pellet is slightly red, repeat this washing step.	250 µl G1 750 µl ddH_2O	1 ml G1 3 ml ddH_2O	2 ml G1 6 ml ddH_2O
5. Add buffer G2 and completely resuspend the pellet by vortexing for 15 – 30 sec. For sample volumes larger than 1 ml please vortex 30 sec at maximum speed. For smaller volumes 15 sec should be enough.	1 ml	5 ml	10 ml
6. Add proteinase K (20 mg/ml) and incubate the mixture for 60 min at 50°C.	50 µl	100 µl	200 µl
Equilibration			
Equilibrate the column with buffer N2.	1 ml	2 ml	5 ml
Adsorption			
Add buffer N2 (room temperature) to the sample. Vortex the mixture for 15 sec at maximum speed. Load the sample onto the column, equilibrated with buffer N2. Allow it to enter the resin by gravity flow. Don't use any additional force (syringes etc.).	1 ml	5 ml	10 ml
Washing			
Wash the column with buffer N3.	3 x 1 ml	3 x 4 ml	3 x 8 ml
Elution			
Elute the genomic DNA with buffer N5. A second elution step with the same volume of elution buffer will increase the yield slightly (15– 20%).	1 ml	5 ml	8 ml
Precipitation			
Add 0.7 volume of isopropanol (room temperature), mix, incubate 30 – 60 min at room temperature and centrifuge at 4°C (~15,000 rpm) for 25 min. If the pellet looks glassy, air dry it (not longer than 15 min) and redissolve it in slightly alkaline buffer (e.g. TE, pH 8) overnight on a shaker or at 55°C for 1 – 2 hours. If a white pellet is obtained, additionally wash it with 70% ethanol and redissolve it as described above.	0.7 ml	3.5 ml	5.6 ml

3.4 Isolation of genomic DNA from bacteria

For the isolation of genomic DNA from bacteria MACHEREY-NAGEL does not offer ready-to-use kits. The columns as well as the buffer solutions can be ordered separately. The NucleoBond® buffer set III (Cat. No. 740 603) contains all necessary buffers (buffers G3, G4, N2, N3, and N5), proteinase K, as well as RNase A. Lysozyme is not included in this buffer set.

Before starting the procedure, please read the remarks on the following page.

Step of the procedure	Special conditions for NucleoBond® columns		
	AXG 20	AXG 100	AXG 500
maximum yield of genomic DNA	20 µg	100 µg	500 µg
culture volume	2 – 4 ml	15 – 20 ml	60 – 80 ml
Cell disruption			
1. Dissolve RNase A in buffer G3 (final concentration 200 µg/ml)			
Dissolve proteinase K in Proteinase Buffer (20 mg/ml). Store buffer G3 and the proteinase K solution at 4 °C. If lysozyme is required, dissolve it in H_2O (100 mg/ml).			
2. Pellet the bacterial cells from an appropriate volume of culture by centrifugation at 3,000 – 5,000 x g for 10 min. Discard the supernatant.			
3. Resuspend the bacterial pellet in buffer G3 by vortexing.	1 ml	4 ml	12 ml
4. Add the lysozyme (optional) and the proteinase K stock solution.	20 µl lysozyme	80 µl lysozyme	300 µl lysozyme
	25 µl proteinase K	100 µl proteinase K	450 µl proteinase K
Incubate the mixture at 37°C for	20 min	40 min	60 min
5. Add buffer G4 and mix by vortexing.	0.4 ml	1.2 ml	4 ml
6. Incubate the mixture at 50°C for 30 min. If the lysate is not clear after incubation with proteinase K the incubation time should be prolonged. If any insoluble cell components are observed, the sample should be clarified by a short centrifugation (5,000 x g, 5 min)			
ATTENTION: It is very important to obtain a clear lysate in order to avoid clogging of the column.			
Equilibration			
Equilibrate the column with buffer N2.	1 ml	2 ml	5 ml

Step of the procedure	Special conditions for NucleoBond® columns		
	AXG 20	AXG 100	AXG 500
Adsorption			
Add buffer N2 (room temperature) to the sample. Vortex the mixture for 15 sec at maximum speed. Load the sample onto the column, equilibrated with buffer N2. Allow it to enter the resin by gravity flow. Don't use any additional force (syringes etc.).	1 ml	5 ml	10 ml
Wash			
Wash the column with buffer N3.	3 x 1 ml	3 x 4 ml	3 x 8 ml
Elution			
Elute the genomic DNA with buffer N5. A second elution step with the same volume will increase the yield slightly (15 – 20%).	1 ml	5 ml	8 ml
Precipitation			
Add 0.7 volume of isopropanol (room temperature), mix, incubate 30 – 60 min at room temperature and centrifuge at 4°C (~ 15,000 rpm) for 25 min. If the pellet looks glassy, air dry it (not longer than 15 min) and redissolve it in slightly alkaline buffer (e.g. TE, pH 8) overnight on a shaker or at 55°C for 1 – 2 hours. If a white pellet is obtained, additionally wash it with 70% ethanol and redissolve it as described above.	0.7 ml	3.5 ml	5.6 ml

Remarks:

Gram-positive bacteria are more difficult to lyse. Reagents like lysozyme, lysostaphin etc. are recommended and compatible with this method. When using clinical samples, tissue or other inhomogenous material for DNA isolation, additional homogenisation techniques (Ultraturrax, Dounce homogenisator etc.) in combination with an enzymatic digest (lyticase, lysozyme, lysostaphin) may be necessary. In general follow our standard protocol for the isolation of genomic DNA from bacteria.
In order to obtain ultrapure DNA as well as a good flow rate of the column an overloading of the column must be avoided!
For the first time it is better to start using a low cell number (AXG 20 –4 x 10^9, AXG100 –2 x 10^{10}, AXG 500 –1 x 10^{11}, cells can be increased stepwise).
If bacteria are used that contain plasmid DNA and genomic DNA start with half of the culture volume recommended for non plasmid containing bacteria.

ATTENTION:

Proteinase K must be dissolved in **Proteinase Buffer** (as indicated on the bottle, see also section 1, Kit contents). The dissolved proteinase K is stable at 4°C for 6 months under these conditions. Divide the solution into small aliquots and store at –20°C if the solution will not be used up during this period. Frozen proteinase K stock solutions are stable up to one year.
Before using the **RNase A** it should be redissolved in buffer G3. After addition of RNase A the buffer G3 and buffer G4 should be stored at 4 °C.
Lysozyme should be redissolved in sterile or ddH_2O (100 mg/ml).The solution should be divided in aliquots and stored at –20 °C.

3.5 Isolation of genomic DNA from tissue

For the isolation of genomic DNA from tissue MACHEREY-NAGEL does not offer ready-to-use kits. The columns as well as the buffer solutions can be ordered separately. Our NucleoBond® buffer set IV (Cat. No. 740 604) contains all buffer solutions (buffers G2, N2, N3, and N5), proteinase K, and RNase A.

Before starting the procedure, please read the remarks on the following page.

Step of the procedure	Special conditions for NucleoBond® columns		
	AXG 20	AXG 100	AXG 500
maximum yield of genomic DNA	20 µg	100 µg	400 µg
maximum amount of tissue	20 mg	100 mg	400 mg
Cell disruption			
1. Dissolve RNase A in buffer G2 (200 µg/ml) Dissolve proteinase K in Proteinase Buffer (20mg/ml). Store buffer G2 and the proteinase K solution at 4°C.			
2. Thoroughly homogenize the tissue mechanically (Ultra Turrax) in buffer G2. Alternatively the tissue can be homogenized with a mortar and pestle under liquid nitrogen. The fine powder is redissolved in buffer G2. **ATTENTION:** Homogenize the tissue as good as possible. This step is very important for the lysis procedure as well as for a good flow rate of the NucleoBond® AXG columns.	2 ml	10 ml	20 ml
3. Transfer the homogenate to a 15 or 50 ml screw cap tube. Add the proteinase K stock solution (20 mg/ml) to the homogenate. Mix well by vortexing (30 sec).	25 µl proteinase K	100 µl proteinase K	450 µl proteinase K
4. Incubate the sample at 50°C for 2 hours. If the lysate is not clear after incubation with proteinase K the incubation time should be prolonged. If any insoluble cell components are observed, the sample should be clarified by a short centrifugation (5,000 x g, 5 min). **Attention:** It is very important to obtain a clear lysate in order to avoid clogging of the column.			
Equilibration			
Equilibrate the column with buffer N2.	1 ml	2 ml	5 ml

Step of the procedure	Special conditions for NucleoBond® columns		
	AXG 20	AXG 100	AXG 500
Adsorption			
Add buffer N2 (room temperature) to the sample. Vortex the mixture for 15 sec at maximum speed. Load the sample onto the column, equilibrated with buffer N2. Allow it to enter the resin by gravity flow. Don't use any additional force (syringes etc.).	1ml	5 ml	10 ml
Wash			
Wash the column with buffer N3.	3 x 1 ml	3 x 4 ml	3 x 8 ml
Elution			
Elute the genomic DNA with buffer N5. A second elution step with the same volume will increase the yield slightly (10 – 15%).	1 ml	5 ml	8 ml
Precipitation			
Add 0.7 volume isopropanol (room temperature), mix, incubate 30 – 60 min at room temperature and centrifuge at 4°C (~ 15,000 rpm) for 25 min. If the pellet looks glassy, air dry it (not longer than 15 min) and redissolve it in slightly alkaline buffer (e.g. TE, pH 8) overnight on a shaker or at 55°C for 1 – 2 hours. If a white pellet is obtained, additionally wash it with 70% ethanol and redissolve it as described above.	0.7 ml	3.5 ml	5.6 ml

Remarks:

In order to achieve a high yield of DNA, the tissue samples have to be stored in liquid nitrogen.
If the tissue sample is treated with 20% glycerol or 20% DMSO, the sample has to be centrifuged. The supernatant contains the glycerol or DMSO and can be discarded.

ATTENTION:

Proteinase K must be dissolved in **Proteinase Buffer** (as indicated on the bottle, see also section 1, Kit contents). The dissolved proteinase K is stable at 4°C for 6 months under these conditions. Divide the solution into small aliquots and store at –20°C if the solution will not be used up during this period. Frozen proteinase K stock solutions are stable up to one year.
After the first use **buffer G2** should be stored at 4°C and has to be equilibrated at room temperature before use.
The **buffer G2/RNase A** mixture is stable for 2 – 3 months at 4°C.

3.6 Isolation of genomic DNA from yeast

For the isolation of genomic DNA from yeast MACHEREY-NAGEL does not offer ready-to-use kits. The columns and the most important buffers can be ordered separately. Our NucleoBond® buffer set III (Cat. No. 740 603) contains all buffer solutions (buffers G3, G4, N2, N3, and N5), proteinase K, and RNase A. Sorbitol buffer as well as lyticase or zymolase stock solution have to be prepared fresh. These two enzymes are not included in the buffer set.

Before starting the procedure, please read the remarks at section 3.5.

Step of the procedure		Special conditions for NucleoBond® columns		
		AXG 20	AXG 100	AXG 500
	maximum of genomic DNA	20 µg	100 µg	500 µg
	amount of cells	10^9	$10^9 – 10^{10}$	$10^{10} – 10^{11}$
	culture volume	3–5 ml	10–20 ml	100–500 ml
Cell disruption				
1.	Harvest the culture (YPD medium) by centrifugation for 10 min at 5.000 x g.			
2.	Resuspend the pellet in sorbitol buffer (1 M sorbitol, 100 mM EDTA, 14 mM ß-mercaptoethanol). Add lyticase or zymolase (1 U/ml stock solution) and incubate at 30°C for 30 min.	600 µl sorb. buffer 60 µl lyticase	3 ml sorb. buffer 300 µl lyticase	15 ml sorb. buffer 1.5 ml lyticase
3.	Centrifuge the mixture for 10 min at 5,000 x g. Resuspend the cell pellet in buffer G3 by vortexing.	1 ml	4 ml	12 ml
4.	Add proteinase K stock solution	25 µl proteinase K	100 µl proteinase K	450 µl proteinase K
	and incubate the mixture at 37°C for	20 min	40 min	60 min
5.	Add buffer G4 and mix by vortexing.	0.4 ml	1.2 ml	4 ml
6.	Incubate the mixture at 50°C for 30 min. If the lysate is not clear after incubation with proteinase K the incubation time should be prolonged. If any insoluble cell components are observed, the sample should be clarified by a short centrifugation (5,000 x g, 5 min) **ATTENTION:** It is very important to obtain a clear lysate in order to avoid clogging of the column.			
Equilibration				
	Equilibrate the column with buffer N2.	1 ml	2 ml	5 ml

Step of the procedure	Special conditions for NucleoBond® columns		
	AXG 20	AXG 100	AXG 500
Adsorption			
Add buffer N2 (room temperature) to the sample. Vortex the mixture for 15 sec at maximum speed. Load the sample onto the column, equilibrated with buffer N2. Allow it to enter the resin by gravity flow. Don't use any additional force (syringes etc.).	1 ml	5 ml	10 ml
Wash			
Wash the column with buffer N3.	3 x 1 ml	3 x 4 ml	3 x 8 ml
Elution			
Elute the genomic DNA with buffer N5. A second elution step with the same volume will increase the yield slightly (15 – 20%).	1 ml	5 ml	8 ml
Precipitation			
Add 0.7 volume of isopropanol (room temperature), mix, incubate 30 – 60 min at room temperature and centrifuge at 4°C (~ 15,000 rpm) for 25 min. If the pellet looks glassy, air dry it (not longer than 15 min) and redissolve it in slightly alkaline buffer (e.g. TE, pH 8) overnight on a shaker or at 55°C for 1 – 2 hours. If a white pellet is obtained, additionally wash it with 70% ethanol and redissolve it as described above.	0.7 ml	3.5 ml	5.6 ml

4 Troubleshooting

If any problems with the preparation arise proceed as follows: In order to get an idea of what has been the problem, please collect the flow-through, the wash and the eluate fraction. Precipitate the fractions and load them on an agarose gel. In combination with this troubleshooting guide this will help to solve your problem. Furthermore don't hesitate to contact the Technical Service of MACHEREY-NAGEL, Tel. +49 (0) 24 21 969-270, Fax +49 (0) 24 21 969-279, or contact us at tech-bio@mn-net.com.

Problem	Possible reason	What is to do
No quantitative adsorption of nucleic acids	Salt concentration of the sample is too high:	Dilute the sample or precipitate and redissolve it.
	pH value of the sample is higher than pH 6.5:	Adjust the pH of the sample.
	Anionic detergents (e.g. SDS) are in the sample:	Remove SDS by filtration with a folded filter. Load the sample onto the column immediately after finishing the sample preparation steps.
	pH or salt concentrations of buffer 1, 2, 3, or 4 are too high:	Adjust pH or prepare new buffers.
	High viscosity sample:	Increase the volume of sample preparation buffers to reduce viscosity.
	Column was overloaded with nucleic acid:	Use a bigger column or purify excess DNA on a new column.
	No nucleic acid in the sample:	Check the pH of all buffers used and repeat the purification
No elution of RNA, dsDNA or ssDNA	No nucleic acid adsorbed:	See above
	Salt concentration or pH of the washing buffer are too high:	Adjust pH or prepare a new buffer.
	Salt concentration of the elution buffer or its pH are too low:	Adjust pH or prepare a new buffer.
Column blocked	Viscosity of the sample is too high:	Use larger buffer volumes for sample preparation. Use a prolonged centrifugation step to get a clear supernatant. Mix the sample with one volume of equilibration buffer.

Problem	Possible reason	What is to do
Oligonucleotide contamination in RNA fraction	Salt concentration of buffer 1 or its pH is too low:	Adjust pH or prepare a new buffer.
DNA contamination in RNA fraction	Salt concentration of buffer 3 or its pH are too high:	Adjust pH or prepare a new buffer.
RNA contamination in DNA fraction	Salt concentration of buffer 3 or its pH are too low:	Adjust pH or prepare a new buffer.
	RNase A digestion was insufficient:	Add more RNase. Increase volume of wash buffer.
No nucleic acid after precipitation	Nucleic acid pellet was lost:	Handle with care.
	Nucleic acid was not resuspended:	Handle with care.
	Nucleic acid was not precipitated:	Check organic solvent. Mix the suspension and use a longer centrifugation time.
Insufficient resuspension of purified nucleic acid	Nucleic acid was overdried:	Dissolve for a longer time at somewhat higher temperature.
	Residual salt or organic solvent in the pellet:	Wash the pellet with an organic solvent of low viscosity.
		Increase buffer volume.
Strong white pellet after precipitation	Coprecipitation of the salt:	Check the purity of the isopropanol.
		Perform precipitation at room temperature (except centrifugation)
		Don't let the eluate drop directly into a vial with isopropanol.
Sequencing problems:		
contaminated DNA	inefficient lysis	The pellet of bacterial cells must be redissolved in buffer S1 quantitatively. Reduce the culture volume or increase the amount of buffers S1, S2 and S3.
degraded DNA	alkaline lysis	Incubation with buffer S2 should not proceed longer than 5 min.

5 Product use restriction / warranty

NucleoBond® RNA/DNA / CB / AXG kit components were developed, designed and sold **for research purposes only**. They are suitable ***for in vitro uses only***. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is rather the responsibility of the user to verify the use of the **NucleoBond® RNA/DNA / CB / AXG** kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

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