Primerdesign™ Ltd

genesig® Easy

DNA/RNA Extraction Kit

50 extractions

Universal kit for isolation of RNA / DNA from food, water, clinical, veterinary and other samples types.

For general laboratory and research use only

DNA Testing

Everything…
Everyone…
Everywhere…

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1 Quick guide

1.1 Quick guide – First steps when you open a new kit

Upon opening a new genesig® Easy DNA/RNA extraction kit, a couple of components need to be mixed to make them ready to use.

No.1a
Proteinase K buffer

Blue pipette
(500µl)

No.1
Proteinase K

Magnetic beads

No.3a
Magnetic Beads

Blue pipette
Move entire
contents

No.3
Binding buffer

Give tube No.3a a vigorous shake to resuspend the beads before transfering the entire contents in to bottle No.3

1.2 Quick guide – sample prep

Depending on your sample type you may need to perform a simple sample prep step to prepare for DNA/RNA extraction.

Meat/Food/Tissue and Genesig® Easy Plant Extractions

Combine

- Approx. 10-20mg of homogenised tissue (a match head in size)
- 500µl (Blue pipette) Sample Prep Solution
- Mix well with pipette
- Let large pieces settle out.
- Use clear top layer of liquid for DNA/RNA extraction

Blood/Serum/Plasma/Other liquids

No sample prep required. Add sample direct to stage 1 of DNA/RNA extraction

Dry Swab samples

Shake swab in 500µl (Blue pipette) Sample Prep Solution
Wait 30 minutes
Squeeze out the swab
Discard swab
Add prepared sample to Step1 of extraction

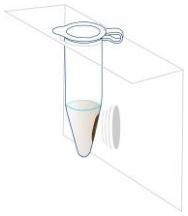
Faeces/Soil

Combine

- Approx. 10-20mg of faeces/soil (a match head in size)
  or 200µl (White pipette) if using liquid faeces
- 500µl (Blue pipette) Sample Prep Solution
  Mix well with pipette
  Leave to settle out
  Use clear top layer of liquid for DNA/RNA extraction

1.3 Quick guide – How to magnetise

The genesig® Easy DNA/RNA extraction kit uses minute magnetic beads to bind to the DNA/RNA in your sample. A magnet is then used to pull the beads out of solution so the DNA/RNA can be separated with ease.

How to magnetise	
Add beads to your sample	
Place your tube in the genesig® magnetic separator rack. Immediately the beads will begin to gather on the wall of the tube	
After 1-2 minutes all the beads will be clumped in one spot.	
Use a pipette to remove the liquid whilst being careful not to disturb the beads.	

1.4 Quick guide – DNA/RNA extraction

genesig® easy DNA/RNA extraction			
Step		Lab-in-a-box pipette	
1	Combine • 200µl sample • (20µl of Tube1*) • 200µl Tube 2 • 10µl Internal extraction control DNA/RNA†		Shake Wait 15 minutes
2	Add • 500µl Tube 3		Shake Wait 5 minutes Magnetise! Remove all liquid
3	Add • 500µl Tube 4		Shake Wait 30 seconds Magnetise! Remove all liquid
4	Add • 500µl Tube 5		Shake Wait 30 seconds Magnetise! Remove all liquid
5	Add • 500µl Tube 6		Shake Wait 30 seconds Magnetise! Remove all liquid Air dry for 10mins with the lid open
6	Add • 200µl Tube 7		Shake Wait 30 seconds Magnetise!
DNA/RNA is in the liquid!			

* Only required when working with Meat/Food/Tissue. Exclude for other sample types.
† If using a standard or advance genesig kit, use 4µl of the internal extraction control template.

2 Components

2.1 Kit contents

genesig® Easy:	Extraction Kit	Plant Extraction Kit	
	Sample Prep Solution	Sample Prep Solution	30ml
Tube No.1	Proteinase K (lyophilized)	Proteinase K (lyophilized)	2 x 6 mg
Tube No.1a	Proteinase K Buffer	Proteinase K Buffer	8 mL
Tube No.2*	**Lysis Buffer**	**Plant Lysis Buffer**	15 mL
Tube No.3	genesig® Easy Beads/Binding buffer mix	genesig® Easy Beads/Binding buffer mix	40 mL
Tube No.3a	genesig® Easy Magnetic Beads	genesig® Easy Magnetic Beads	1.5ml
Tube No.4	Wash Buffer 1	Wash Buffer 1	40 mL
Tube No.5	Wash Buffer 2	Wash Buffer 2	40 mL
Tube No.6	80% Ethanol	80% Ethanol	30 ml
Tube No.7	Elution Buffer	Elution Buffer	13 mL

* The contents of this tube differ between the genesig® Easy Extraction and genesig® Easy Plant Extraction kits.

3 Product information

3.1 The basic principle

The **genesig® Easy Extraction** and **genesig® Easy Plant Extraction** kits are designed for the isolation of DNA/RNA from a huge range of sample types (The **genesig® Easy Plant Extraction** kit is specifically for use with plant samples). This kit provides reagents and magnetic beads for isolation of 50 samples of approx 100–200µL. The procedure is based on the reversible adsorption of nucleic acids to magnetic beads under appropriate buffer conditions. Sample lysis is achieved by incubation with a Lysis Buffer containing chaotropic ions supported by Proteinase K digestion as and when required. For binding of nucleic acids to the magnetic beads, Binding Buffer and the genesig® Easy Extraction Beads are added to the lysate. After magnetic separation, the magnetic beads are washed to remove contaminants and salts using Wash Buffers 1 and 2 and 80% ethanol. Residual ethanol from the previous wash step is removed by air-drying. Finally, highly pure DNA/RNA is eluted with low-salt Elution Buffer or water. Purified DNA/RNA can directly be used for downstream applications. The genesig® Easy Extraction kits can be used either manually or automated on standard liquid handling instruments or automated magnetic separators.

3.2 Kit specifications

The **genesig® Easy Extraction** and **genesig® Easy Plant Extraction** kits are designed for rapid manual and automated small-scale preparation of DNA/RNA (the **genesig® Easy Extraction** kit is suitable for use with rom cell-free body fluids such as serum or plasma samples, blood samples or homogenized tissue suspensions, food, broth and many other sample types; the **genesig® Easy Plant Extraction** kit is suitable for use with homogenized plant material suspensions). The kits are designed for use with genesig® Easy magnetic separator or other magnetic separation systems. Manual time for the preparation of 16 samples is around 25 minutes. The purified DNA/RNA can be used directly as template for qPCR.

Automated extraction systems. genesig® Easy Extractions allow easy automation on common liquid handling instruments or automated magnetic separators. The actual processing time depends on the configuration of the instrument and the magnetic separation system used. Typically, 96 samples can be purified in less than 30 minutes using the genesig® Easy Extraction kits on the automation platform.

3.3 Handling of beads

Distribution of beads

A homogeneous distribution of the magnetic beads is essential for a high sample-to-sample consistency. Therefore, before adding the beads to the magnetic beads buffer, make sure that the beads are completely re-suspended. Shake the storage bottle well or place it on a vortexer for a short length of time. Premixing magnetic beads with the binding buffer allows easier homogenous distribution of the beads to individual samples.

Magnetic separation time

The genesig® Easy magnetic separator has been designed to give ideal separation of the magnetic beads from the sample solution. Complete separation of beads from solution occurs within a 1-2 minute time frame. However, if using an alternative automated separation system the attraction of the magnetic beads to the magnetic pins depends on the strength of the magnetic pins, the selected separation plate, distance of the separation plate from the magnetic pins, and the volume to be processed. The individual times for complete attraction of the beads to the magnetic pins should be checked and adjusted on each system. It is recommended to use the separation plates or tubes specified by the supplier of the magnetic separator.

Washing the beads

Washing the beads can be achieved by shaking or mixing. Ensure that the beads are completely detached from the tube wall. A complete wash is only possible once all beads are back in suspension. If using an automated separation system, mixing by shaker or magnetic mixing allows simultaneous mixing of all samples. This reduces the time and number of tips needed for the preparation. Re-suspension by pipetting up and down, however, is more efficient than mixing by a shaker or magnetic mix.

3.4 Elution procedures

Purified DNA/RNA can be eluted directly with the supplied Elution Buffer. Elution can be carried out in a volume of ≥ 50µL. It is essential to cover the genesig® Easy Beads completely with elution buffer during the elution step as the beads must be re-suspended completely. The volume of dispensed elution buffer depends on the magnetic separation system (e.g., the position of the pellet inside the separation plate). For some separators, high elution volumes might be necessary to cover the whole pellet.

4 Storage conditions and preparation of working solutions

Attention:

Some of the Buffers contain chaotropic salt. Wearing gloves and goggles is recommended.

All components of the **genesig® Easy Extraction** and **genesig® Easy Plant Extraction** kits should be stored at room temperature (18–25°C) and are stable for up to one year.

All buffers are delivered ready-to-use.

Magnetic beads:

Give tube No.3a a vigorous shake to resuspend the beads before transfering the entire contents in to bottle No.3

When using the proteinase K, prepare the following:

Add 500µl of tube No.1a to both of the tubes labelled No.1 to re-suspend the Proteinase K. Dissolved Proteinase K solution should be stored at – 20°C ideally in small aliquots

5 Protocols

5.1 Preparation of sample materials

a) Animal / Plant tissue samples (plant samples should be processed with the **genesig® Easy Plant Extraction** kit only)

Homogenize tissue samples. Typically 10–20 mg sample material can be homogenized in 500µL Sample Prep Solution by mixing with a pipette tip or using a bead based homogenizer. If necessary, higher amounts of sample material can be used (up to 25 mg). It should be considered that the co-purified total nucleic acid may cause inhibition in the subsequent PCR assays. After homogenization of the tissue, allow to settle or centrifuge and use up to 200µL clear supernatant for the extraction protocol. If using less than 200µL, adjust with Sample Prep Solution to a final volume of 200µL.

For isolation of RNA:

Tissue samples can also be disrupted in a buffer containing chaotropic salt and beta-mercaptoethanol or TCEP reducing agent.

b) Blood and serum/plasma samples

A sample volume of 100-200µL blood can be added directly to Step 1 of the protocol. Do not use higher volumes. When using less than 200µL samples, adjust with Sample Prep Solution to 200µL.

c) Swab samples

Incubate the swabs with Sample Prep Solution, sodium chloride, or cell culture medium for 30 min with occasional shaking. Remove and squeeze out the swab. Proceed with 200 µL of the particle-free buffer or medium for the extraction protocol.

d) Faeces

Mix 1 volume of faeces (e.g., 200 µl) with 500µl of Sample Prep Solution. Mix vigorously by shaking for 1 min. Allow the particles to settle down or centrifuge with low speed (e.g., at 500 x *g*). For difficult to lyse bacteria, mechanical disruption or treatment using suitable glass beads may be required. Take the supernatant and use 200µL for the extraction protocol.

For difficult to extract faecal samples use 1 volume of faeces (approximately 20mg or 200µl if using liquid faeces) and add 200µl of lysis buffer directly to the sample. Add 20µl of proteinase K and incubate at room temperature for 15 minutes, Transfer the lysate to a new Eppendorf tube leaving behind any particulate matter before adding the magnetic beads. Continue the extraction as normal following this step.

e) TRIzol® lysis

For sample materials such as semen, a TRIzol® lysis may be required. Homogenize 10–30 mg tissue with 1 mL TRIzol® reagent to manufacturer's instructions. After phase separation by centrifugation, remove aqueous, colourless (upper) phase (approximately 400µL). For further processing, start with step 2 of the extraction protocol by mixing 500µL of the aqueous phase with 500µL magnetic bead/binding buffer mix.

5.2 Universal kit for isolation of RNA / DNA from food, water, clinical, veterinary and other samples types

Preparation of sample material

A 200µL sample volume is recommended as standard.

Detailed protocol

This protocol is for manual use with a genesig® magnetic separator and serves as a guideline for adapting the kit to robotic instruments.

1	**Lyse sample** In a 1.5ml flipcap tube: Pre-dispense 200µL of sample to a suitable reaction tube. Add **200µL Lysis Buffer** to the reaction tube. (Add **20 µL Proteinase K** n.b. not required when working with blood/serum/plasma) Mix well by repeated pipetting up and down and incubate at room temperature for 15min. Following the lysis incubation, tap the sample down or spin down in a centrifuge if available to collect any sample from the lysis tube lids.

2	**Bind nucleic acid to magnetic beads** Add **500µL magnetic beads/binding buffer** to the lysed sample. Mix well by shaking then wait 5 minutes *Be sure to mix the genesig® Easy Extraction Beads well before removing them from the storage bottle. Vortex or shake the storage bottle briefly until a homogenous suspension has been formed.* Separate the magnetic beads from the sample by placing the tube in to a magnetic separator. Wait at least 2min until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting. *Do not disturb the attracted beads while aspirating the supernatant.*
3	**Wash with Wash Buffer 1** Remove the tube from the magnetic separator. Add **500µL Wash Buffer 1** and re-suspend the beads by shaking until the beads are re-suspended completely. Then wait 30 seconds. Alternatively, re-suspend beads completely by repeated pipetting up and down. Separate the magnetic beads from the sample by placing the tube in to a magnetic separator. Wait at least 2min until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting. *Do not disturb the attracted beads while aspirating the supernatant*.
4	**Wash with Wash Buffer 2** Remove the tube from the magnetic separator. Add **500µL Wash Buffer 2** and re-suspend the beads by shaking until the beads are re-suspended completely. Then wait 30 seconds. Alternatively, re-suspend beads completely by repeated pipetting up and down. Separate the magnetic beads from the sample by placing the tube in to a magnetic separator. Wait at least 2min until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting. *Do not disturb the attracted beads while aspirating the supernatant.*
5	**Wash with 80% ethanol** Remove the tube from the magnetic separator. Add **500µL 80% ethanol** and re-suspend the beads by shaking until the beads are re-suspended completely. Then wait 30 seconds. Alternatively, re-suspend beads completely by repeated pipetting up and down. Separate the magnetic beads from the sample by placing the tube in to a magnetic separator. Wait at least 2min until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting. *Do not disturb the attracted beads while aspirating the supernatant*.
6	**Air-dry magnetic beads** Air-dry the magnetic bead pellet for 10 min at room temperature with the tube lid open. The beads should be free from any visible liquid ethanol but not left to completely dry out.
7	**Elute DNA/RNA** Remove the tube from the magnetic separator. Add desired volume of **Elution Buffer (50–200µL)** to the tube and re-suspend the beads by shaking. Then wait 30 seconds. Alternatively, re-suspend beads completely by repeated pipetting up and down. Separate the magnetic beads from the sample by placing the tube in to a magnetic separator. Wait at least 2min until all the beads have been attracted to the magnets. Transfer the supernatant containing the purified RNA/DNA to a 0.5ml flipcap tube for storage or use in down stream applications.

	Do not disturb the attracted beads while aspirating the supernatant.

6 Using alternative magnetic systems

6.1 Using alternative magnetic separation systems

When using the **genesig® Easy Extraction** and **genesig® Easy Plant Extraction** kits, the use of the genesig® Easy magnetic separator is recommended. Separation is carried out in individual micro-centrifuge tubes. However, the kit can also be used with other common separators.

Static magnetic pins

Separators with static magnetic pins: This type of separator is recommended in combination with a suitable microplate shaker for optimal mixing of the beads during the washing and elution steps. Alternatively, beads can be mixed in the buffer by pipetting up and down several times. For fully-automated use on liquid handling workstations, a gripper tool is required, the plate is transferred to the magnetic separator for separation of the beads and transferred to the shaker module for re-suspension of the beads.

Movable magnetic systems

Separators with moving magnetic pins: Magnetic pins/rods are moved from one side of the well to the other and vice versa. The beads follow this movement and are thus pulled through the buffer during the wash and elution steps. Separation takes place when the system stops.

Automated separators

Separators with moving magnets: Magnetic beads are transferred into suitable plates or tubes. The beads are re-suspended from the rod-covered magnets. Following binding, washing or elution the beads are collected again with the rod-covered magnets and transferred to the next plate or tube.

6.2 Adjusting the shaker settings

If using a plate shaker for the washing and elution steps, the speed settings have to be adjusted carefully for each specific separation plate and shaker to prevent cross-contamination between wells. Proceed as follows:

Adjusting shaker speed for binding and wash steps:

Load 600µL of dyed water to the wells of the separation plate. Place the plate on the shaker and start shaking with a moderate speed setting for 30 seconds. Turn off the shaker and check the plate surface for small droplets of dyed water.

Increase speed setting, shake for an additional 30 seconds, and check the plate surface for droplets again.

Continue increasing the speed setting until you observe droplets on top of the separation plate. Reduce speed setting, check again, and use this setting for the washing steps.

Adjusting shaker speed for the elution step:

Load 100µL of dyed water to the wells of the collection plate and proceed as described above.

7 Safety instructions

The following components of the **genesig® Easy Extraction** and **genesig® Easy Plant Extraction** kits contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section.

7.1 GHS classification

Harmful components do not need to be labelled with H and P phrases until 125mL or 125g.

Component	Hazard contents	GHS symbol	Hazard phrases	Precaution phrases
Lysis buffer	Guanidine hydrochloride 36-50 %	Warning	H302, H315, H319	P280, P301+P312, P302+P352, P305+P351+P338, P330, P333+P313, P337+P313
Binding buffer	Sodium perchlorate 20–40 % + ethanol 35–55 %	Warning	H226, H302	P210, P233, P301+P312, P330
Wash buffers 1 and 2	Sodium perchlorate 5–20 % + ethanol 20–35 %	Warning	H226	P210, P233
Proteinase K	Proteinase K, lyophilized	Danger	H315, H319, H334, H335	P261,P280, P304+P341, P305+P351+P338, P342+P311
Ethanol	Ethanol 80%	Danger	H226	P210

Hazard phrases	
H226	Flammable liquid and vapour.
H302	Harmful if swallowed.
H315	Causes skin irritation.
H319	Causes serious eye irritation.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335	May cause respiratory irritation.

Precaution phrases	
P210	Keep away from heat/sparks/open flames/hot surfaces. No smoking.
P233	Keep container tightly closed.
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.

P301+P312	IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P304+P341	IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing.
P305+P351+P338	IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing
P330	Rinse mouth.
P333+P313	If skin irritation or a rash occurs: Get medical advice/attention.
P337+P313	If eye irritation persist. Get medical advice/attention.
P342+P311	If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician.

8 Appendix

8.1 Troubleshooting

Problem	Possible cause and suggestions
Poor yield/low sensitivity	*Insufficient elution buffer volume* Beads pellet must be covered completely with elution buffer. *Insufficient performance of elution buffer during elution step* Remove residual buffers during the separation steps completely. Remaining buffers decrease the efficiency of the following wash and elution steps. *Beads dried out* Do not let the beads dry as this might result in lower elution efficiencies. *Aspiration of attracted bead pellet* Do not disturb the attracted beads while aspirating the supernatant, especially when the magnetic bead pellet is not visible in the lysate. *Aspiration and loss of beads* Time for magnetic separation too short or aspiration speed too high.
Low purity/ low sensitivity	*Insufficient washing procedure* Make sure that beads are re-suspended completely during the washing procedure. If shaking is not sufficient to re-suspend the beads completely mix by repeated pipetting up and down.
Poor performance of RNA in downstream applications	*Carry-over of ethanol from wash buffers* Be sure to remove all of the 80% ethanolic wash solution from the final wash, as residual ethanol interferes with downstream applications. *Ethanol evaporation from wash buffers* Close buffer bottles tightly to avoid ethanol evaporation from bottles.
Carry-over of beads	*Time for magnetic separation too short* Increase separation time to allow the beads to be completely attracted to the magnet before aspirating any liquid from the well. *Aspiration speed too high (elution step)* High aspiration speed during the elution step may cause bead carry-over. Reduce aspiration speed for elution step.

8.2 Ordering information

Visit ***www.genesig.com*** for more detailed product information.

8.3 Product use restriction / warranty

genesig® Easy Extraction and **genesig® Easy Plant Extraction** kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original PRIMERDESIGN product leaflets.

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