RapidGenomics
Genotyping Solutions

QuickExtract DNA Extraction Solution

Manual

QuickExtract™ DNA Extraction Solution is part of the Epicentre™ product line, known for its unique genomics kits, enzymes, and reagents which offer high quality and reliable performance.

LGC Diagnostics & Genomics

Contents

1. Introduction to QuickExtract chemistry

The QuickExtract DNA Extraction Solution provides a fast, simple and inexpensive method to extract nucleic acids for PCR and/or for RT-PCR. The procedure will extract prokaryotic, eukaryotic and viral nucleic acids. This solution has been used for a wide variety of sample types including, but not limited to, liquid samples, cells in culture, tissues, organs, insects, hair follicles, fish scales and swabs.

QuickExtract DNA Extraction Solution is available in multiple dispense volumes. The optimal ratio of QuickExtract Solution to sample may be determined empirically and may be readily scaled up or down depending on sample type and size.

The standard protocol consists of two heating steps and is completed within a single microcentrifuge tube or well of a microtiter plate. It does not involve bind and release steps from a column or matrix. Depending upon the nature of the sample, an initial grinding or homogenization step may be employed and/or a preincubation step with appropriate lytic enzyme(s) may be performed prior to the two heating steps.

2. Kit contents and storage conditions

Catalogue number	Part number	Volume supplied	Storage conditions	Number of extractions**
QE0905T	SS000035-1	5 mL	-20 °C*	Up to 100
QE09050	SS000035-2	50 mL	-20 °C*	500 to 1,000
QE0901L	SS000035-3	1000 mL	-20 °C*	10,000 to 20,000

Table 1. QuickExtract DNA Extraction Solution product codes, volumes and storage conditions.
*Freezer should not have a defrost cycle.
** Assumes 50 µL to 100 µL per Extraction. Volume of QuickExtract DNA Extraction Solution per sample may be scaled up or down depending upon sample type, mass or volume. Optimal ratio of QuickExtract DNA Extraction Solution per sample may be determined empirically.

Minimise the number of freeze-thaw cycles. Thawed solution can be stored at 4 °C for 1 month or refrozen in smaller aliquots. For ordering information please refer to our website.

3. Quality Control

QuickExtract DNA Extraction Solution is function-tested by assaying for the production of a PCR product from a human X chromosomal marker (Cooperative Human Linkage Center, marker DXS7132) using a buccal cell DNA preparation as template.

4. Experimental procedure

4.1 General information before starting

Be certain enough QuickExtract DNA Extraction Solution is used to sufficiently cover the sample. Depending upon the sample type it may be necessary to perform a brief centrifugation ensure the sample is completely immersed in the QuickExtract DNA Extraction Solution.

The second heating step at 95-98 °C may deform some plastics. If using microtiter plates be sure that the plates you intend to use are suitable for this temperature.

4.2 General information before starting

Some sample types may contain inhibitors of enzymes used for downstream processing which can impact the applications of the extracted nucleic acids. Environmental and metagenomic samples may have lignins and polyphenols from the surrounding environment, and tissues and organ samples can have significant amounts of heme, all of which are efficient inhibitors of PCR.

If amplification is unsuccessful using undiluted extract, test a dilution series (1 in 4, 1 in 8, 1 in 16, and 1 in 32) of the processed sample to determine the best balance between nucleic acid concentration and PCR inhibitors. While it may seem counterintuitive to use less starting nucleic acid material, better results are sometimes achieved by using less processed sample; the amount of potential enzyme inhibitors in the reaction is reduced whilst still having sufficient nucleic acid as template for the reaction.

4.3 Sample grinding/mincing

Optimal nucleic acid release requires access to the individual cells and it is therefore important to determine whether mincing or grinding is required for your sample type.

- Tail snips: these should be minced.
- Tissue (e.g. slices, biopsy samples, organ fragments): these can benefit from grinding to disperse cells from connective tissue. When tissue is in the QuickExtract DNA Extraction Solution use a micropestle for grinding or, alternatively, grinding may be done in liquid nitrogen.
- Insects: transfer the insect to a microcentrifuge tube, add the QuickExtract DNA Extraction Solution and use a micropestle for grinding.

4.4 Pre-incubation with lytic enzymes

Determine if preincubation with lytic enzyme(s) is required for your sample type.

- Gram positive bacteria: lysis and nucleic acid release may be markedly enhanced for many gram-positive bacteria by a preincubation with **Ready-Lyse™ Lysozyme** (Cat. No. R1804M).
- Samples with known cell wall linkages: employ the appropriate lytic enzyme (mutanolysin, lysostaphin, etc.) to partially digest the cell wall linkages.
- Metegenomic samples: consider preincubation with Metapolyzyme (Cat. No. MAC4L, Sigma-Aldrich) which is a cocktail of 6 lytic enzymes.
- Insects: sample grinding in the presence of chitinase, plus pre-incubation with chitinase, may improve nucleic acid release.

4.5 High throughput considerations

4.5.1 Volume and addition of QuickExtract solution

Ensure that a sufficient volume of QuickExtract solution is used to enable safe removal of processed sample without aspirating debris.

If using a water bath that holds plates on their side, care needs to be taken to ensure the QuickExtract DNA Extraction Solution covers the sample.

When adding QuickExtract DNA Extraction Solution to the sample, it should be added above the well so that the same tips can be used to dispense multiple plates without cross-contamination.

4.5.2 Presence of debris

Some samples can contain debris which may plug the tips of automated liquid handling devices. A brief centrifugation step may be required to sediment debris thus enabling the processed sample to be aspirated without disturbing and co-aspirating the debris.

4.5.3 Heating step temperature

When performing high-throughput applications, it may not be practical to perform the second heating step at 98 °C as some microtiter plates will warp at this high temperature. This step may be performed at 95 °C. Ensure that the plates you intend to use are suitable for this temperature.

4.5.4 Downstream dispensing of extracted nucleic acids

If extracted samples are later dispensed using a liquid handler, we recommend observing for pipetting issues. Depending on the sample source, bubbles may form in the tips during tip conditioning and lead to dropouts. This may be resolved by making the pipette wash more stringent and limiting the conditioning step.

4.6 Extraction protocols

In this section you will find specific protocols for a range of different sample types.

Note that typical volume of QuickExtract DNA Extraction Solution per sample is from 50 uL to 100 uL, the amount used may be scaled up or down as necessary, depending upon sample type and size.

4.6.1 Swab

Collect swab sample (buccal, vaginal, rectal, wound, skin, any hard surface) using swab of choice. LGC recommends a swab with a plastic shaft and a foam pad as the collection device, rather than a bristle brush or cotton swab.. The swab may be processed immediately or stored at -20 °C or lower for subsequent processing.

If storing or transporting the collected sample, air dry the swab for 10-15 minutes at room temperature and then place in the original packaging. Store the dry swab at -20 °C or lower in the original packaging for up to 6 months.

Nucleic acid yield is directly correlated with the amount of starting material on the swab. If yield is not a concern, use only one swab; if yield must be maximised, use multiple swabs.

1. Place the collection end of the swab into a tube containing a sufficient volume of QuickExtract DNA Extraction Solution to cover the sample collection part of the swab. Rotate the swab a minimum of five times while pressing the swab against the inside of the tube. Continue this action while removing the swab from the tube to ensure most of the liquid remains in the tube.
2. Secure the cap on the tube tightly and vortex the tube for 15 seconds to disperse cells.
3. Incubate the tube at 65 °C for 6 minutes.
4. Vortex the tube for 15 seconds.
5. Transfer the tube to 95-98 °C and incubate for 2 minutes.
6. Vortex the tube for 15 seconds.
7. Centrifuge the tube containing processed sample at top speed in a microcentrifuge to sediment any debris.
8. Remove 1 µL of processed sample to serve as template in a 10 µL total volume PCR (or RT-PCR) reaction.
9. Store the processed sample at -20 °C for up to 6 months or at -70 °C for longer term storage.

4.6.2 Gram negative bacteria

If working with single colonies on plates: use a sterile loop to transfer a single colony to 50-100 µL QuickExtract DNA Extraction Solution. The optimal volume of QuickExtract DNA Extraction Solution should be determined empirically.

If working with liquid culture: transfer 100 µL culture to a 1.5 mL microcentrifuge tube and spin for 1 minute. Remove the bulk of the liquid above the pelleted cells, leaving approximately 20 µL of liquid in the tube, and resecure the cap. Vortex mix for 15 seconds to disperse cells. Add an equal volume (approximately 20 µL) of QuickExtract DNA Extraction Solution to the tube.

1. Secure the cap on the tube tightly and vortex the tube for 15 seconds to disperse cells.
2. Incubate the tube at 65 °C for 6 minutes.
3. Vortex the tube for 15 seconds.
4. Transfer the tube to 95-98 °C and incubate for 2 minutes.
5. Vortex the tube for 15 seconds.
6. Centrifuge the tube containing processed sample at top speed in a microcentrifuge to sediment any debris.
7. Remove 1 µL of processed sample to serve as template in a 10 µL total volume PCR (or RT-PCR) reaction.
8. Store the processed sample at -20 °C for up to 6 months or at -70 °C for longer term storage.

4.6.3 Gram positive bacteria, yeast, fungi, metagenomic samples

These samples can have cell walls that are difficult to breach. It may be necessary to perform a preincubation with an appropriate lytic enzyme or cocktail of enzymes prior to combining the sample with the QuickExtract DNA Extraction Solution (see section 4.4).

1. Add sample to a microcentrifuge tube and pellet cells by centrifugation for 1 minute.
2. Carefully remove supernatant.
3. Add 50 µL of sterile PBS or equivalent and vortex the tube for 15 seconds to disperse cells.
4. Add appropriate lytic enzyme(s) and mix by vortexing for 15 seconds.
5. Incubate for 15 minutes at 37 °C (optimal time and temperature determined empirically).
6. Add an equal volume of QuickExtract DNA Extraction Solution and mix by vortexing for 15 seconds.
7. Incubate the tube at 65 °C for 6 minutes.
8. Vortex the tube for 15 seconds.
9. Transfer the tube to 95-98 °C and incubate for 2 minutes.
10. Vortex the tube for 15 seconds.
11. Centrifuge the tube containing processed sample at top speed in a microcentrifuge to sediment any debris.
12. Remove 1 µL of processed sample to serve as template in a 10 µL total volume PCR (or RT-PCR) reaction.
13. Store the processed sample at -20 °C for up to 6 months or at -70 °C for longer term storage.

4.6.4 Tissue samples (including organs or biopsy samples)

These samples will typically require grinding for optimal release of nucleic acids.

1. Add 1-5 mg sample to a microcentrifuge tube containing 50-100 uL of sterile PBS or equivalent isotonic buffer solution.
2. Use a micropestle to grind the sample.
3. Add an equal volume of QuickExtract DNA Extraction Solution.
4. Secure the cap on the tube tightly and vortex the tube for 15 seconds to disperse cells.
5. Incubate the tube at 65 °C for 6 minutes.
6. Vortex the tube for 15 seconds.
7. Transfer the tube to 95-98 °C and incubate for 2 minutes.
8. Vortex the tube for 15 seconds.
9. Centrifuge the tube containing processed sample at top speed in a microcentrifuge to sediment any debris.
7. Remove 1 µL of processed sample to serve as template in a 10 µL total volume PCR (or RT-PCR) reaction.
8. Store the processed sample at -20 °C for up to 6 months or at -70 °C for longer term storage.

4.6.5 Tail snip

Transfer a 0.5-1 cm section of a mouse tail snip, finely diced using a fresh blade, to a microcentrifuge tube containing 50-100 µL of QuickExtract DNA Extraction Solution. The optimal volume of QuickExtract DNA Extraction Solution should be determined empirically.

1. Secure the cap on the tube tightly and vortex the tube for 15 seconds to disperse cells.
2. Incubate the tube at 65 °C for 6 minutes.
3. Vortex the tube for 15 seconds.
4. Transfer the tube to 95-98 °C and incubate for 2 minutes.
5. Vortex the tube for 15 seconds.
6. Centrifuge the tube containing processed sample at top speed in a microcentrifuge to sediment any debris.
7. Remove 1 µL of processed sample to serve as template in a 10 µL total volume PCR (or RT-PCR) reaction.
8. Store the processed sample at -20 °C for up to 6 months or at -70 °C for longer term storage.

4.6.6 Hair, feather or fish scale

Transfer a 0.5-1 cm region of a hair with follicle, a 0.5-1 cm quill-end of breast feather, or a fish scale to a microcentrifuge tube containing ~50 µL of QuickExtract DNA Extraction Solution. The optimal volume of QuickExtract DNA Extraction Solution should be determined empirically. For some sample types, it may be beneficial to grind the sample using a micropestle.

1. Secure the cap on the tube tightly and vortex the tube for 15 seconds to disperse cells.
2. Incubate the tube at 65 °C for 6 minutes.
3. Vortex the tube for 15 seconds.
4. Transfer the tube to 95-98 °C and incubate for 2 minutes.
5. Vortex the tube for 15 seconds.
6. Centrifuge the tube containing processed sample at top speed in a microcentrifuge to sediment any debris.
7. Remove 1 µL of processed sample to serve as template in a 10 µL total volume PCR (or RT-PCR) reaction.
8. Store the processed sample at -20 °C for up to 6 months or at -70 °C for longer term storage.

4.6.7 Insect

Transfer the insect to a microcentrifuge tube. Add 100 µL sterile PBS or equivalent to the tube and grind using a micropestle. Addition of chitinase may be required at this stage (see section 6.4).

If chitinase is added, incubate samples using the optimised time and temperature (determined empirically). Spin the microcentrifuge tube briefly to pellet sample debris.

Carefully aspirate and transfer a 50 µL aliquot to a microcentrifuge tube containing 50 µL of QuickExtract DNA Extraction Solution.

1. Secure the cap on the tube tightly and vortex the tube for 15 seconds to disperse cells.
2. Incubate the tube at 65 °C for 6 minutes.
3. Vortex the tube for 15 seconds.
4. Transfer the tube to 95-98 °C and incubate for 2 minutes.
5. Vortex the tube for 15 seconds.
6. Centrifuge the tube containing processed sample at top speed in a microcentrifuge for 1 minute to sediment any debris.
7. Remove 1 µL of processed sample to serve as template in a 10 µL total volume PCR (or RT-PCR) reaction.
8. Store the processed sample at -20 °C for up to 6 months or at -70 °C for longer term storage.

4.6.8 Saliva, mucus, serum, urine

Transfer 50-100 µL of sample to a microcentrifuge tube. Add an equal volume of QuickExtract DNA Extraction Solution.

1. Secure the cap on the tube tightly and vortex the tube for 15 seconds to disperse cells.
2. Incubate the tube at 65 °C for 6 minutes.
3. Vortex the tube for 15 seconds.
4. Transfer the tube to 95-98 °C and incubate for 2 minutes.
5. Vortex the tube for 15 seconds.
6. Centrifuge the tube containing processed sample at top speed in a microcentrifuge for 1 minute to sediment any debris.
7. Remove 1 µL of processed sample to serve as template in a 10 µL total volume PCR (or RT-PCR) reaction.
8. Store the processed sample at -20 °C for up to 6 months or at -70 °C for longer term storage.

4.6.9 Cells in liquid culture - non adherent

Liquid culture refers to $1x10^3$ to $1x10^5$ mammalian cells.

1. Transfer 100 µL of liquid culture to a microcentrifuge tube.
2. Pellet the cells by centrifugation at 1000 x g at 4 °C for 5 minutes.
3. Carefully aspirate the bulk of the culture media.
4. Add 50 µL sterile PBS (or equivalent isotonic solution).
5. Vortex mix to resuspend and evenly disperse cells.
6. Add 50 µL of QuickExtract DNA Extraction Solution to the cells and vortex mix for 1 full minute.
7. Incubate the tube at 65 °C for 6 minutes.
8. Vortex the tube for 15 seconds.
9. Transfer the tube to 95-98 °C and incubate for 2 minutes.
10. Vortex the tube for 15 seconds.
11. Centrifuge the tube containing processed sample at top speed in a microcentrifuge for 1 minute to sediment any debris.
12. Remove 1 µL of processed sample to serve as template in a 10 µL total volume PCR (or RT-PCR) reaction.
13. Store the processed sample at -20 °C for up to 6 months or at -70 °C for longer term storage.

4.6.10 Cells in liquid culture - adherent

Liquid culture refers to $1x10^3$ to $1x10^5$ mammalian cells.

1. Carefully aspirate the culture media from the tube(s) or well(s) of a microtiter plate.
2. Add 50 µL of sterile PBS (or equivalent isotonic solution) to the tube or well and vortex for 15 seconds to disperse cells..
3. Add 50 µL of QuickExtract DNA Extraction Solution to the cells and vortex mix for 1 full minute.
4. Incubate at 65 °C for 6 minutes.
5. Vortex mix for 15 seconds.
6. Transfer the tube or plate to 95-98 °C and incubate for 2 minutes.
7. Vortex the tube for 15 seconds.
8. Centrifuge the tube containing processed sample at top speed in an appropriate centrifuge for 1 minute to sediment any debris.
9. Remove 1 µL of processed sample to serve as template in a 10 µL total volume PCR (or RT-PCR) reaction.
10. Store the processed sample at -20 °C for up to 6 months or at -70 °C for longer term storage.

5. Troubleshooting

For optimal release of nucleic acids, it is important to have cells evenly dispersed in the QuickExtract DNA Extraction Solution. Although for many samples this may be achieved simply by vortexing, some samples require mincing, grinding or inclusion of a preincubation with an appropriate lytic enzyme. Please review the information in sections 4.3 and 4.4 for more guidance.

Optimisation of PCR may be necessary. Follow common practices for optimisation (e.g. titrate magnesium concentration, adjust annealing and/or elongation temperature and time, add PCR enhancers). Alternatively, the **FailSafe™ PCR PreMix Selection Kit** (Cat. No. FS99060) provides a quick optimisation procedure to increase success rates.

6. Further support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com.

7. References

Below are some representative references demonstrating the use of QuickExtract chemistry for many different sample types.

Sample Type	Representative Reference
Swab	Analyzing DNA from buccal cells is a reliable method for the exclusion of cystic fibrosis. Results of a pilot study. Genet Med 8, 175-177 (2006)
Gram positive bacteria / yeast/ fungi / metagenomic samples	Identification and prevalence of tetracycline resistance in enterococci isolated from poultry in Ilishan, Ogun State, Nigeria. J Pharm Bioallied Sci. 2016 Jan-Mar;8(1):69-73
Tissue samples	Mouse liver: CRISPR/Cas9 engineering of adult mouse liver demonstrates that the Dnajb1-Prkaca Gene fusion is sufficient to induce tumors resembling fibrolamellar hepatocellular carcinoma. Gastroenterology. 2017 Dec;153(6):1662-1673.e10
Tail snip	The Multicopper Ferroxidase Hephaestin Enhances Intestinal Iron Absorption in Mice. PLoS ONE 2014, 9(6)
Hair / feather / fish scale	Fish scale: Marine migration behaviour of brown trout from five watercourses flowing into a common fjord system in Northern Norway. Norwegian University of Science and Technology, 2018
Insect	Rapid, large-scale species discovery in hyperdiverse taxa using 1D MinION sequencing. BMC Biol 17, 96 (2019)
Saliva / mucus / serum / urine	Saliva: Point-of-care testing for COVID-19 using SHERLOCK diagnostics. medRxiv [Preprint]. 2020
Cells in liquid culture	Enhanced bacterial immunity and mammalian genome editing via RNA polymerase-mediated dislodging of Cas9 from double strand DNA breaks. Mol Cell. 2018 July 05; 71(1): 42-55.e8

Table 2. QuickExtract references for a broad range of starting sample types.

There are multiple references demonstrating that nucleic acids extracted using QuickExtract DNA Extraction Solution are suitable for downstream RT-PCR. See below for seminal articles and protocols.

1. One-step RNA extraction for RT-qPCR detection of 2019-nCoV. Monica Sentmanat, Evguenia Kouranova, Xiaoxia Cui. bioRxiv 2020.04.02.022384

2. Evaluating the efficacy of RT-qPCR SARS-CoV-2 direct approaches in comparison to RNA extraction. Israeli, Ofir et al. International Journal of Infectious Diseases, Volume 99, 352-354

3. A 5-min RNA preparation method for COVID-19 detection with RT-qPCR. Alim Ladha, Julia Joung, Omar O. Abudayyeh, Jonathan S. Gootenberg, Feng Zhang. medRxiv 2020.05.07.20055947

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