

Nucleic acid extraction and purification technologies

AMBIHITH I



# Extraction and purification solutions from LGC Biosearch Technologies

Biosearch Technologies is unique in the nucleic acid extraction and purification marketplace, in that we are both a service provider, and a developer of proprietary chemistries and instrumentation. We develop chemistries and instrumentation for customers to use in their own laboratories, and it is these same products which we use in each of our service laboratories. This allows Biosearch Technologies to be highly flexible, and offer individual solutions for each of our customers.

To provide the most efficient and effective extraction or purification method, we offer not only a range of different technologies, but also the capability to develop tailor-made kit systems for all applications and downstream requirements.

Our range of technologies allows delivery of optimised nucleic acid extractions and purifications (e.g. genomic DNA, plasmids, RNA) from an unrivalled array of matrices including:

- Plant material (including leaves, seeds and other plant matrices)
- Livestock samples (including blood, animal tissue, hair follicle, buccal swabs and saliva)
- Human samples (including blood, tissue and forensic samples)
- Bacteria, prokaryotes and other microorganisms.

- 3 DNA/RNA extraction chemistries
  QuickExtract™
  PCR-grade extraction
- 4-7 DNA and RNA purification chemistries sbeadex™ Lightning next generation sequencing-grade purifications
  - Total nucleic acid purification chemistries

    MasterPure™
  - **9** Automation
- Purification services

  DNA purification services
- Ordering information
- 12 Overview



DNA/RNA extraction chemistries

#### QuickExtract

#### **PCR-grade extraction**

For DNA screening and genotyping applications, PCR-grade genomic DNA is sufficient, and can be rapidly and efficiently extracted using QuickExtract DNA Extraction Solution. In 3-8 minutes, and using only a single-tube protocol, PCR-grade genomic DNA or RNA from almost any sample type can be extracted, without the use of centrifugation, spin columns or any toxic organic solvent. (figure 1).

The QuickExtract method allows for the inexpensive processing of one to hundreds of samples simultaneously and is also compatible with robotic automation (figure 1).



Heat at 65 °C for 6 minutes and 98 °C for 2 minutes



Figure 1. Procedure for obtaining PCR-ready DNA using QuickExtract DNA Extraction Solution

The <u>range of QuickExtract Extraction Solutions</u> for additional sample types includes:

- QuickExtract DNA Extraction Solution
- QuickExtract FFPE DNA Extraction Solution
- QuickExtract Plant DNA Extraction Solution
- QuickExtract RNA Extraction Solution

QuickExtract Solution has been used to rapidly extract DNA and RNA from samples such as hair follicles, quill-end cells of feathers, cell/tissue cultures, buccal cells, zebrafish organs and scales, and mouse tail snips. The extracted DNA and RNA is suitable for PCR and RT-PCR analyses (figure 2), such as genomic, transgenic, or viral DNA/RNA screening in animals, or for genetic or environmental research and screening in humans, plants and other organisms (e.g. CRISPR genome editing analysis).

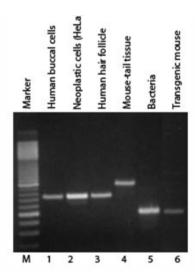


Figure 2. Samples extracted with QuickExtract DNA Extraction Solution. PCR was performed using primers to amplify the regions indicated: Lanes 1-3, human  $\beta$ -globin; lane 4, transgenic mouse GAPDH; lane 5, *E. coli* 16S ribosomal RNA gene; lane 6, transgenic SV40 T antigen.

# DNA and RNA purification chemistries

#### sbeadex next generation sequencing-grade purifications

The requirement to isolate high-quality DNA and RNA in high yields for PCR/RT-PCR, cloning, sequencing/next generation sequencing (NGS) and other applications can be met by Biosearch Technologies' sbeadex chemistries which is a technical improvement of our classical mag<sup>TM</sup> beads chemistry.

All sbeadex kits use surface-modified superparamagnetic particles that bind nucleic acids via a novel, two-step binding mechanism (figure 3). Firstly, the nucleic acids affix to the beads via polar interactions, and secondly, the nucleic acids are bound via an affinity-driven mediator. This second binding step allows the final wash steps to be carried out using water-based wash solutions, eliminating the need for a drying stage, and minimising any contaminating ethanol carryover into the final eluate. The switch between binding mechanisms also allows for greater purity of nucleic acids.

#### The advantages of sbeadex include:

- High-quality DNA/RNA: suitable for all genomic applications (including NGS)
- Automation: compatible with Biosearch Technologies' automated oKtopure<sup>™</sup> and other open platforms (e.g. KingFisher<sup>™</sup> Flex)
- Flexibility: tailor-made lysis conditions and protocols according to customer requirements
- Configuration: flexible batch sizes and kit component volumes
- Higher purity: no organic solvents such as ethanol in final wash buffer
- Salt-free eluates and high OD<sub>260/230</sub>

sbeadex beads	Specification
Colour	Dark brown
Superparamagnetic bead structure	Proprietary double-coated technology (competitor: single coated)
Size and shape	Irregular shape, 5-10 µm (diameter)
Maximum capacity	>2 µg DNA/mg sbeadex particle
Binding material and technology	2-step binding mechanism: first silica and second DNA adaptor
Purification format	96 or 384 (depending on automation)
Automation compatibility	Applicable with common open liquid handlers (e.g. KingFisher, Tecan, Hamilton, Beckman-Coulter)
Sample material	Include blood, plant, livestock, forensic, cell/tissue, bacterial, viral and fungal samples
Application	PCR, KASP™, BHQ™ Probe chemistry, Sanger, NGS, Microarrays
Storability	12 months

Table 1. Features and specification of sbeadex beads

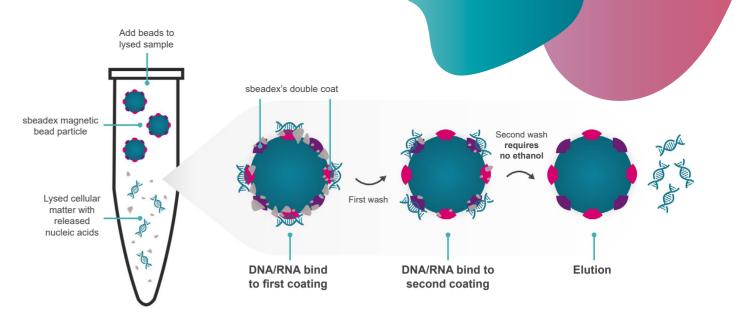


Figure 3. Standard workflow for all sbeadex-based purification chemistry

#### sbeadex Lightning



Our new sbeadex Lightning product line is the long awaited solution for a time- and cost-saving nucleic acid purification that has all advantages of our sbeadex chemistry – in a significantly reduced workflow that also reduces costs.

It provides a revolutionary, game-changing approach to isolate high-quality nucleic acids in high yields with a superfast and simple 3-step protocol (excluding lysis) - purifying DNA in as little as 5 minutes from a variety of lysed plant and animal sample materials.

It therefore clearly increases sample throughput per day – in both manual and automated nucleic acid purification.

Our new sbeadex Lightning is not only faster, simpler and more convenient – but also more environmentally friendly.

Fewer protocol steps means less buffers/solutions, less consumables, less waste, packaging and transportation energy/cost which all helps to protect the environment. The reagents of the sbeadex Lightning chemistry are not only reduced in volume but are also less hazardous than common reagents used in classical magnetic bead-, spin column- or solution-based workflows.

We provide sbeadex Lightning in a new, flexible kit format that allows a plant and livestock sample-specific optimisation of the purification protocol: A core kit (containing our sbeadex particles, the proprietary binding buffer and an elution buffer) that is completed by individual components (e.g. different lysis buffers) determined by using the <a href="mailto:sbeadex Lightning Nucleic Acid Purification Starter Kit for plant and livestock samples">samples</a>.

For cell purification try our new <u>sbeadex Lightning Cell</u> DNA Kit.

Original sbeadex chemistry:

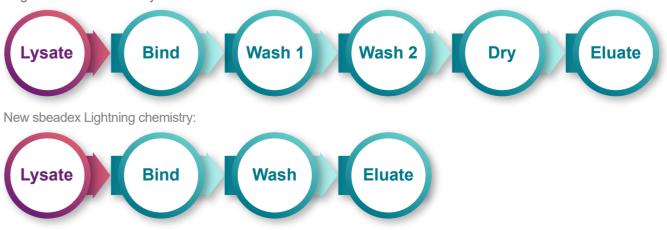


Figure 2. Different to current magnetic beads or spin column-based purification protocols, sbeadex Lightning has a simple 3-step and 5-minute protocol (bind-elute-wash).



#### **Plant DNA**

Plant samples are often challenging due to secondary metabolites, high levels of sugars and polyphenols or robust cell walls. sbeadex Plant DNA Purication Kit protocols for leaf and/or seed material from a broad range of plant species have been validated and can be automated on all open platforms, including the Biosearch Technologies' oKtopure. sbeadex purification protocols tailored to specific customer needs and optimised for distinct plant species have been successfully established for many plant species, e.g.: grains, vegetables, oilplants, fruits and others - list available online.

Kit	Amount of starting material	Sample material	Typical DNA yields
sbeadex Mini Plant DNA Purification Kit	5-10 mg lyophilised or 10-30 mg fresh tissue	Plant leaves and seeds	~2 µg
sbeadex Maxi Plant DNA Purification Kit	10-30 mg lyophilised or 40-120 mg fresh tissue	Plant leaves and seeds	~10 µg

Table 2. Specification of available sbeadex plant kits

#### **Livestock DNA**

The majority of NGS-based genomic analysis technologies (including those working with livestock samples) typically require high-quality DNA preparations, which are often challenging and labour-intensive to produce. The sbeadex Livestock DNA Purification Kit is based on one protocol which can be used for all sample types - and the oKtopure can be used for all livestock samples (excl. blood).

Sample type	Animal species
Blood* Hair follicle Saliva Semen Tissue (e.g. ear punch) Buccal swab FTA cards	Bovine Chicken Dog Fish Goat Horse Sheep Pig

Table 3. Sample types from different species applicable with the sbeadex Livestock DNA Purification Kit.

sbeadex Lightning Nucleic Acid Purification Kits are suited for a variety of plant and livestock sample materials – and we are continuously testing new samples. The protocols can be easily adapted for different automation platforms (e.g. KingFisher, Hamilton Star, Biomek or the Tecan liquid handlers).

While there is no loss in DNA quality and yield, sbeadex Lightning shows considerable cost and time savings compared to our original sbeadex chemistry (and of course all the traditional mag beads workflows). It also scores with reduced plastic consumables, waste, packaging and shipping/storage costs - helping to protect the environment.

Amount of starting material	Typical DNA yields	Time	Consum- ables	Liquid waste
Plant: 10-30 mg of lyo- philised or 40-120 mg of fresh tissue	Plant: Up to 10 μg from 15 mg leaves or 50 mg seed	Up to 90% time saving*	Up to 9-times less plastics*	Up to 7-times less liquid waste*

Table 4. Specifications and savings when using sbeadex Lightning technology for purification of DNA from plant samples.

A list of sample materials that we have tested with sbeadex Lightning is available online.

#### Cell DNA



#### sbeadex Lightning Cell DNA Kit

sbeadex Lightning Cell DNA Kit is suitable for animal and human cell culture. The sbeadex Lightning technology purifies high-quality DNA from cells in a simple 4-step process, typically requiring only 11 minutes\* from sample to eluate.

#### **Blood DNA**

Blood samples can often vary considerably in quality and this can lead to wide variations in the consistency and yield of extracted DNA, should be a sh Blood DNA Purification Kit provides high yields of high-molecular weight, double-stranded DNA from different blood preparations including those treated with anticoagulants, including:

- EDTA blood
- · Heparin blood
- Citrate blood
- · Buffy coat preparations.

Kit application areas	Amount of starting volume	Sample material	Typical DNA yields
sbeadex Blood DNA Purification Kit	200 μL	Whole blood, buffy coat, EDTA/heparin/ citrate blood	4-13 μg

Table 5. Specifications of available sbeadex blood kits

#### Pathogen DNA and RNA

The sbeadex Pathogen Nucleic Acid Purification Kit allows automation-friendly high-throughput, high-quality DNA and RNA purifications from swab (stored in UTM), sputum/saliva, blood, urine, stool, plasma, serum and cerebrospinal fluid (CSF). Please find a complete list of pathogens and matrices tested so far on our website.

#### The advantages of sbeadex Pathogen Nucleic **Acid Purification Kit include:**

- Qualified protocol for RUO Covid-19 assays (qualified for research use only using SeraCare's AccuPlex™ reference material)
- Compatible with most popular robotic platforms, including the KingFisher and our high-throughput oKtopure instrument
- Fast both short (<20 min) and standard</li> (20-40 min) protocols available
- High-throughput batch sizes of >5,000 purifications per day
- · Water-based wash buffers reduce solvent carryover, resulting in absence of enzyme inhibitors in final eluate
- High-quality nucleic acids for all downstream applications including qPCR, RT-qPCR and NGS.

Kit application areas	Amount of starting volume	Sample material	Typical DNA/RNA yields
sbeadex Pathogen Nucleic Acid Purification Kit	100 µL (200 µL with oKtopure)	Swab, sputum/ saliva, blood, urine, stool, plasma, serum, CSF	4-12 μg

Table 6. Specifications of available sbeadex blood kits

#### Further sbeadex nucleic acid purification kits

- sbeadex Forensic DNA Purification Kit
- sbeadex Tissue DNA Purification Kit
- sbeadex Plasmid DNA Purification Kit
- sbeadex PCR Clean-Up Kit
- sbeadex SAB Kit

For more information, please see our webpage.

<sup>\*</sup> Not compatible with the oKtopure

sbeadex Lightning for plants and livestock

<sup>\*</sup> In comparison to commonly used competitor kits and depending on the protocol (manual/automated using particular robotic platforms)

<sup>\*</sup> refers to incubation times only and includes cell lysis

# Total nucleic acid purification chemistries

#### **MasterPure**

Some applications require flexibility of isolating high-molecular weight genomic DNA, total cellular RNA or total nucleic acid from the same sample. The MasterPure Complete DNA and RNA Purification Kit allows for isolation of NGS-grade DNA, RNA or total nucleic acid in 30-60 minutes using a single kit, and is suitable for many different sample types.

Applications for the MasterPure kit include:

- Library preparation for next generation sequencing of genomic DNA and RNA
- DNA methylation studies (e.g. with use with the Illumina<sup>®</sup> Infinium<sup>®</sup> HumanMethylation BeadChips) Genomic DNA and cDNA cloning

- PCR, qPCR, RT-PCR and qRT-PCR
- Microarray analysis (including CGH and gene expression profiling)

The kit uses a scalable salt-precipitation protocol which eliminates the need for hazardous chemicals (no need for phenol, chloroform or guanidine). Depending on the amount of nucleic acid required and size of the sample, different MasterPure variants are available, including:

- MasterPure Complete DNA and RNA Purification Kit (basic kit version)
- MasterPure DNA Purification Kit for Blood
- MasterPure Gram Positive DNA Purification Kit
- MasterPure Yeast DNA or RNA Purification <u>Kits.</u>

### **Automation**

One example for the need of high-throughput molecular biology-based technologies is for plant and livestock breeders to accelerate their breeding programmes. Throughput, DNA quality, down-stream application requirements, required labour and running costs are the main drivers and

deciding factors when selecting high-throughput automation platforms. Biosearch Technologies can offer the oKtopure to meet these requirements, which allows for standardised, (semi) automated, high-throughput DNA purifications (table 6).

Key features	oKtopure
Grade of automation	Fully automated
Throughput	Up to 5,000 samples/day
Format	96-well
Applications	Nucleic acid isolation from plant samples (e.g. leaf, seed), livestock samples (e.g. ear punch, tissue), samples of pathogenic microorganisms (e.g. SARS-CoV2, influenza) etc.
DNA quality	Very high (NGS-grade)
Downstream applications	All genomics-based applications, including NGS
Replicator functions	96-well
Dimensions (cm)	170 x 68.5 x 65
Laboratory requirements	Air pressure
Consumables	Tip wash option

Table 7. Key features of oKtopure automated platform, compatible with the sbeadex chemistry

#### **Large volume DNA purifications**

Biobanking and other human research markets require large volume purification technologies which provide high quality DNA. Biosearch Technologies offers a cost-effective, easy and rapid methods for the purification of DNA from large volume samples (table 6).

Features	mag PLUS XL
Technology	DNA cell-components are enriched, and residual proteins are digested away. The DNA is subsequently precipitated, washed, dried, and then resuspended
Automation	Manual purifications only
Applications	NGS-grade
Starting volume	Scalable: up to 10 mL
Sample types	Whole blood, saliva (including Oragene collection tubes), tissue
Typical DNA yields	10-800 µg

Table 6. Key features of large volume DNA purification chemistries



# Purification services\*

We at Biosearch Technologies have developed purification for DNA protocols from a wide range of starting materials and volumes which enable the delivery of services for most applications or custom solutions utilising our unique range of inhouse technologies.

## DNA purification services

We have developed highly-optimised <u>DNA</u> <u>purification service</u> protocols, starting from a wide variety of sample types, including:

- Plant (roots, leaves, seeds etc.)
- Blood (human, animal, FTA cards, buffy coat)
- Tissue (hair, saliva, liver, brain etc.)
- Tissue/cell culture
- Forensic samples
- · Bacterial cell pellets.

Feature	Customer benefit
Flexible high-throughput laboratories utilising our oKtopure systems.	Applicable for any project size and sample type.
High quality nucleic acid with optimal yield from column-based methods and superior purity of nucleic acid using magnetic bead-based methods.	Efficient purification of high quality DNA with the highest quantity of DNA possible.
20+ years of experience in DNA extraction and genotyping.	Trustworthy, robust, reproducible and reliable data.
Standard and rapid sample turnaround time options.	Turnaround times ranging from 2-8 weeks depending on the project needs.
Convenient services like sample management, sample storage, quantification and normalisation, genotyping, NGS and more.	Additional customisable services to suit your needs.

Table 8. Key features of Biosearch Technologies purification services

### Ordering information

Purification – superparamagnetic beads – sbeadex*		
Part number	Preparations	Description
NAP41601	96	sbeadex Mini Plant DNA Purification Kit
NAP41602	96	sbeadex Maxi Plant DNA Purification Kit
NAP41405	96	sbeadex Tissue DNA Purification Kit
NAP44401	96	sbeadex Blood DNA Purification Kit
NAP40-024-01	96	sbeadex Pathogen Nucleic Acid Purification Kit
NAP44701	96	sbeadex Livestock DNA Purification Kit
NAP41301	96	sbeadex Plasmid DNA Purification Kit
NAP41501	96	sbeadex Forensic DNA Purification Kit
NAP41701	96	sbeadex PCR Clean-Up Kit
NAP41151	10 mL	Debris capture beads
NAP45001	5 mL	sbeadex SAB Kit (sequencing application beads)
Purification – s	superparamagne	tic beads – mag*
NAP40401	96	mag mini DNA purification kit
NAP40402	96	mag midi DNA purification kit
NAP40403	10	mag maxi DNA purification kit
NAP40404	10	mag maxi PLUS DNA purification kit
NAP40601	96	mag plant DNA purification kit
NAP40501	96	mag forensic DNA purification kit
NAP40701	96	mag nanogram DNA purification kit
NAP40810	10	PLUS XL manual DNA purification kit
Total nucleic a	cid purification c	hemistries - MasterPure
MC89010	10	MasterPure Complete DNA &
MC85200	200	RNA Purification Kit
MPY80200	200	MasterPure Yeast DNA Purification Kit
MPY03100	100	MasterPure Yeast RNA Purification Kit
MGP04100	100	MasterPure Gram Positive DNA Purification Kit
MB711400	For 400 mL blood	MasterPure DNA Purification Kit for Blood Version II

* Other sizes (e.g. 960 preparations or bulk amoun	ts) as well as customised
versions are also available. Please see our website	for more information.

Purification – superparamagnetic beads – sbeadex* Lightning		
Part number	Preparations	Description
NAP40-032-00	20	sbeadex Lightning Nucleic Acid Purification Starter Kit
NAP40-030-01	100	sbeadex Lightning
NAP40-030-02	1000	Nucleic Acid Purification Core Kit A
NAP40-031-01	100	sbeadex Lightning
NAP40-031-02	1000	Nucleic Acid Purification Core Kit B
NAP40-033-01	96	sbeadex Lightning NEW
NAP40-033-10	960	Cell DNA Kit

Please see links above for more information and for required components (lysis buffers) and optional components (Protease K solution, debris capture beads).

DNA/RNA extraction - QuickExtract				
Part number	Volume	Description		
QE0905T	5 mL	QuickExtract DNA		
QE09050	50 mL	Extraction solution		
QEP70750	50 mL	QuickExtract Plant DNA Extraction solution		
QER090150	50 mL	QuickExtract RNA Extraction solution		
QEF81050	50 mL	QuickExtract FFPE DNA Extraction solution		

Other sizes (e.g. bulk amounts) and customised versions (e.g. a lyo-compatible version) are also available. Please see our website for more information.

Automation – oKtopure				
Part number	Description			
KBS-0009-001	oKtopure high-throughput DNA extraction robot			
KBS-0009-002	oKtowash™, concentrated wash buffer (500 mL)			
KBS-0009-003	oKtopure offline tip wash option			
KBS-0009-004	oKtopure mix plates (Thermo 1.2 mL deep well plate)			
KBS-0009-005	Wash buffer bulk reservoirs (pack of 4)			
KBS-0009-999	Extended on-site fully inclusive service contract			
KBS-0010-003	oKtopure tips			
KBS-6212-027	Magnetic plate			

Please find more information on our oKtopure automation

#### LGC

### Overview

	QuickExtract	<u>sbeadex</u>	mag	<u>MasterPure</u>
Key features	3-8 minute quick and easy extraction technology for PCR- grade DNA	Magnetic beads with flexible protocols and no chaotrophic salts/alcohol in final wash buffer	Enable efficient and high quality purification of small and large DNA fragments from different samples	DNA and RNA purification from same workflow
Mechanism of action	Chemically based release of nucleic acids and inactivation of PCR inhibitors	Surface-modified superparamagnetic particles	Modified superparamagnetic particles	Salt precipitation-based chemistry
Products	QuickExtract DNA Extraction Solution QuickExtract FFPE DNA Extraction Solution QuickExtract Plant DNA Extraction Solution QuickExtract RNA Extraction Solution	sbeadex Lightning Cell DNA Kit sbeadex Lightning Nucleic Acid Purification Kit sbeadex Plant DNA Purification Kit sbeadex Livestock DNA Purification Kit sbeadex Blood DNA Purification Kit sbeadex Tissue DNA Purification Kit sbeadex Forensic DNA Purification Kit sbeadex Plasmid DNA Purification Kit sbeadex Plasmid DNA Purification Kit sbeadex Pathogen Nucleic Acid Purification Kit sbeadex PCR clean-up Kit sbeadex SAB Kit	mag mini kit mag midi kit mag maxi kit mag maxi PLUS kit mag plant kit mag forensic kit mag nanogram kit PLUS XL manual kit	MasterPure Complete DNA and RNA Purification Kit  MasterPure DNA Purification Kit for Blood  MasterPure Gram Positive DNA Purification Kit  MasterPure Yeast RNA Purification Kit  MasterPure Yeast DNA Purification Kit
Automation potential	Fully-automated (heating required)	Fully automated (excluding lysis)	Fully automated (excluding lysis)	Fully-automated (centrifugation required)
Corresponding instrumentation	Liquid handler	oKtopure and all open platform liquid/magnetic handlers	Liquid/magnetic handler	Liquid handler
Scalability	96-well format	96-well format	96-well format	96-well format
Options for customisation	Adaption of volume	Adaption to any sample material (lysis)	Adaption of volume and to any sample material (lysis)	Adaption of volume

Table 9. Overview of extraction and purification chemistries from Biosearch Technologies



biosearchtech.com

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