

Risk assessment RA14 Nucleic acid extraction

<https://sports sciencesafety.stir.ac.uk>

Faculty / Service Area	Faculty of Health Sciences and Sport	Location	Analytical Lab 4B140, Teaching laboratories 2U
Description of work task / equipment /area being assessed			
<p>Nucleic acid extraction: The extraction of DNA and RNA from cells and tissues samples is a fundamental procedure for molecular biology work. Clinical samples employed in Sport Sciences include saliva, blood and skeletal and adipose tissue of human origin. This risk assessment (RA) covers DNA and RNA extraction and the key procedures in the initial cell disruption and subsequent nucleic acid purification from the crude lysate using commercial kits and standard procedures. See also RA85 Analytical Lab.</p>			
Change log	<p>16 Oct 2016 GD Version 1.1 29 Aug 2022 New format Version 1.2 16 Aug 2023 KLB RA amended for all nucleic acid extraction methods</p>		
Head of faculty	Prof Jayne Donaldson	Safety officer	Dr Nidia Rodriguez Sanchez
Completed by	Kerry Bartie	Date	16 th August 2023
Reviewed by	Dr Nidia Rodriguez Sanchez	Date	25 th Aug 2023
	Chris Grigson	Date of next review	August 2024
Equipment used	Centrifuges, fume hood, homogeniser, spectrophotometer, Qubit reader		
Categories of people involved	Staff, UG, PG, Visitors		
Duration of activity	1 day	Frequency of activity	Daily
Legal compliance to standards and regulations required	<p>Health and Safety at Work act 1974 (HASAWA) https://www.hse.gov.uk/legislation/hswa.htm</p> <p>Management of Health and Safety at Work Regulations 1999 (MHSWR) https://www.legislation.gov.uk/ukSI/1999/3242/contents/made</p>		

Provision of Work Equipment Regulations 1998 (PUWER)
<https://www.hse.gov.uk/work-equipment-machinery/puwer.htm>

The Control of Substances Hazardous to Health Regulations 2004 (COSHH)
<https://www.hse.gov.uk/coshh/>

Special Waste Amendment (Scotland) Regulations 2004 SSI 112
<https://www.legislation.gov.uk/ssi/2004/112/contents/made>

The Hazards

*Details under relevant heading in appendix

All hazards other than category F.6 (Chemical and biological hazards) should go here

Faculty / Service Area:			FHSS, PENRG		Location:		Cottrell Room 4B140		
Description of work task / equipment /area being assessed:									
Postgraduate and departmental research analysis									
What are the hazards?	Hazard category	Who might be harmed and how?	What are you already doing to control the risks?	*Risk rating	What additional controls (if any) are required to reduce the risks?	*Risk rating	Action by who?	Action by when?	Date of completion
<p>Failure to use lab equipment safely</p> <p>Fume hood</p>	F4 Working with equipment	<p>Lab users/operator</p> <ul style="list-style-type: none"> Exposure to toxic and corrosive substances 	<ul style="list-style-type: none"> Instruction and SOP Ensure fan is in operation before starting work. Always work with hatch at the recommended height safety level Keep fume hood clean 	Low	Yearly inspection and maintenance service (Estates & Campus services)	Low	Competent person	Continual	

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Centrifuge	F4 Working with equipment	Individual lab users <ul style="list-style-type: none"> Liquid items not balanced correctly Secure and tighten lids Contamination of internal compartment and lid if a spillage/leak occurs	Instruction, SOP and equipment manuals Protective PPE <ul style="list-style-type: none"> Lab coat and gloves Spillage procedure <ul style="list-style-type: none"> Containing spill and use of cleaning agents 	Low	User log sheet Yearly inspection and maintenance service	Low	Competent person		
Homogeniser	F4 Working with equipment	Homogenisation requires the use of hazardous reagents Improper use could lead to spillages	Instruction, SOP and equipment manuals Protective PPE <ul style="list-style-type: none"> Lab coat and gloves Spillage procedure <ul style="list-style-type: none"> Contain spill, remove contaminated items to fume hood, dispose of hazardous waste 		No lone working with phenol				

COSHH Hazards

Anything in category F.6 (Chemical and biological hazards) should go here

*Details under relevant heading in appendix










Manufacturers COSHH data sheets are required for all chemical hazards and should be attached

The Substance What are the hazards and *classification? *Route of exposure	*WEL mg/m3	Who might be harmed and how?	What are you already doing to control the risks?	*Risk rating	What additional controls (if any) are required to reduce the risks?	*Risk rating	Action by who?	Action by when?	Date of completion	Health monitoring
Lysis reagents: SDS EDTA Enzymes: Proteinase K DNase RNase Buffers: Tris Salts: Sodium chloride Sodium acetate Irritant		Investigators Contact with skin	Instruction, SOP, manufacturers protocols, MSDS Wear gloves, lab coat and eye protection Eye wash station	Low	Weigh out SDS in fume hood Clean area after use		Competent person			

<p>Guanidinium isothiocyanate Harmful Irritant</p>		<p>Investigators Contact with skin Formation of hazardous compounds with bleach</p>	<p>PPE as above Avoid contact with oxidising agents</p>	<p>Low</p>	<p>Work in small volumes</p>					
<p>Mercaptoethanol Toxic Bromo-chloropropane Harmful Phenol Toxic Corrosive</p>		<p>Investigators Contact with skin</p>	<p>PPE as above Conduct work in fumehood Do not dispose of waste down the drain Dispose of unused product and contaminated items as segregated hazardous waste for specialist disposal</p>		<p>Training in proper use of homogeniser to reduce risk of leakage No lone working with phenol</p>					

Isopropanol		Investigators	PPE as above		Avoid naked flames					
Ethanol		Contact with skin	Store in flammables cupboard		Use hazard label					
Flammable					Use bottle carrier transporting Winchesters					
<p>References</p> <p>DNA collection kit from saliva https://www.dnagenotek.com/us/products/collection-human/oragene-discover/500-series/OGR-500.html</p> <p>Extraction of DNA from saliva using prepIT.L2P and Oragene collection kit https://www.dnagenotek.com/us/pdf/PD-HB-00002.pdf</p> <p>QIAzol Lysis reagent handbook https://www.qiagen.com/gb/resources/download.aspx?id=61c3ddb-d-69c1-4b68-ab89-a428f14a9245&lang=en</p> <p>RNeasy Plus Kit https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/rna-purification/total-rna/rneasy-plus-kits</p>										

Append supplier safety data sheets for all substances here:

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QIAzolLysisReagent.pdf
- 
590-RNeasyFibrous TissueMiniKit(50)-en
- 
EDTA_SDS.pdf
- 
mercaptoethanoISD S.pdf
- 
BromoChloroPropa neSDS.pdf
- 
OrageneDNA_SDS.pdf
- 
PrepITL2P_SDS.pdf
- 
sodiumDodecylSulf ate_SDS.pdf
- 
trizmaBaseSDS.pdf

Standard operating procedure

Procedure:

Wear gloves and lab coat throughout procedure. For phenol, BCP and mercaptoethanol manipulations conduct work in the fume hood during working hours.

DNA extraction:

DNA extraction kits are commercially available for processing of saliva samples (DNA Genotek Oragene Kit), blood and tissue (Qiagen). Alternatively, in-house protocols can be developed. Wear gloves, lab coat and eye protection as lysis and purification reagents contain skin irritants. Protocols are available from the manufacturer and should be followed closely taking note of any reagent incompatibilities. Protein removal can either be by salt precipitation and clarification of the supernatant, or by capture of the DNA within silica columns or magnetic beads. Addition of alcohol (ethanol or isopropanol) precipitates the DNA, with washing in 80% ethanol removing excess salts before elution in Tris buffer pH 8.0 or molecular grade water.

RNA extraction:

Lysis reagents (QIAzol, Trizol Lysis Reagent, RLT Buffer) containing guanidine thiocyanate and phenol are used to isolate high quality RNA (as well as DNA and proteins) from cell and tissue samples. Within our laboratories these samples are most notably of human origin and in particular blood and skeletal muscle samples. Kits using silica bead columns for RNA purification are also available. Bromo-chloro-propane can be used as a phase separation reagent.

Read and understand the manufacturer's safety data sheets and the protocol before starting work. These reagents contain guanidine thiocyanate, which can form highly reactive compounds and toxic gasses when combined with bleach or strong acids. Keep away from oxidizing agents, and acidic or alkaline products at all times.

Always work with QIAzol Lysis reagent or Trizol in a fume hood and during normal working hours. Wear a lab coat, gloves and safety glasses for protection. Avoid direct contact with the reagent as contact to skin, eyes, or respiratory tract may cause chemical burns to the exposed area. If contact to skin or eyes occurs, immediately wash the exposed area with copious amounts of water for 15 minutes and seek medical attention if necessary. On the event of inhalation of vapours, move to fresh air and seek medical attention, if necessary.

If liquid containing this reagent is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area **first** with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Do not dispose QIAzol Lysis, Trizol reagent and bromo-chloro-propane down the drain. Contaminated items should be treated as hazardous waste for specialist disposal.

Nucleic acid quantification:

DNA and RNA samples can be quantified on the spectrophotometer or by Qubit dye readings. Wear gloves when handling nucleic acid samples to avoid nucleases.