

Laboratory Safety Guideline Qiagen and Other Nucleic Acid Kits

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Overview

Many labs use nucleic acid extraction kits, such as those made by Qiagen, Zymo, Mo Bio, and Invitrogen, to isolate ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) from a wide variety of samples, including animal and plant cells and tissue, bacteria, and yeast. This document outlines minimum expectations for use of nucleic acid extraction kits in Harvard labs. Departments or labs may choose to implement more stringent requirements for those operating in their spaces.

Hazards

Hazard Symbol	Hazard Description
	Qiagen Kits can have multiple hazards depending on the chemical composition of the specific kit's reagents. Please review the manufacturer's Safety Data Sheet (SDS) and additional SDSs and
	chemical information resources.

Phenol-Containing Kits

Some kits designed to isolate RNA contain phenol, such as the Qiagen miRNeasy Kit and RNeasy Lipid Tissue Mini Kit. In Qiagen kits, the phenol-containing solution is called Qiazol.

Phenol is a highly toxic and corrosive chemical that is designated as a Particularly Hazardous Substance (PHS). Phenol is readily absorbed through the skin, causes localized chemical burns, and can cause systemic effects

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including central nervous system, liver, and kidney damage leading to severe illness or death. Toxic or fatal amounts of phenol can be absorbed through relatively small areas of skin. Initial skin exposure may go unnoticed due to a numbing effect.

When feasible, choose RNA extraction kits that are phenol-free.

 If using a kit that contains Qiazol, refer to <u>Lab Safety Guideline: Phenol</u> for detailed information on phenol handling and emergency procedures.

Bleach Reactions

Most nucleic acid extraction kits that do not contain phenol are low hazard on their own. However, mixing components of those kits with incompatible chemicals can produce high hazard by-products and gases. Specifically, a number of incidents have been reported concerning researchers mixing bleach (sodium hypochlorite) with kit waste. Some of these kits contain guanidine salts, such as guanidine thiocyanate and guanidine hydrochloride, that may produce hazardous gases when combined with bleach or strong acids. Other components are also incompatible with bleach.

Please reference Appendix 1: Bleach Incompatibility and Appendix 2: Qiagen Kit Components.

Training

Lab personnel working with nucleic acid extraction kits must complete applicable EHS training and keep it up to date.

- General Lab Safety: Renewed annually.
- <u>Laboratory Safety Orientation Checklist</u>: Completed for each lab a person works in and kept on file by the lab.
- <u>Laboratory Biosafety</u>: Renewed annually.

In addition, kit users should review this document and be familiar with emergency procedures.

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Precautions

Personal Protective Equipment

Proper personal protective equipment (PPE) and attire are important whenever working with hazardous chemicals. Each space should have a lab-specific PPE Assessment posted for reference by lab users.

The following table outlines basic PPE requirements. More information can be found on Lab PPE.

For phenol-containing kits, refer to the PPE section of Lab Safety Guideline: Phenol.

PPE Type	Requirement
Attire	Wear a combination of clothing and shoes that fully cover the legs and feet.
Eye and Face	Wear safety glasses with side shields at a minimum if kits have components with acute or
Protection	chronic toxicity, including phenol. Refer to the SDS for information on the specific hazards of the kit's chemicals.
	Use safety goggles when there is a greater risk of splashes and for spill cleanup.
Gloves	Wear compatible chemically resistant gloves when handling potentially hazardous
	chemicals, including nucleic acid kits. Refer to each chemical's SDS and Lab Glove Selection
	Guide for help identifying compatible gloves.
Lab Coat	Lab coats are required when handling phenol-containing solutions, when handling more
	than minimal amounts of chemicals with acute or chronic toxicity, when working in
	Biosafety Level 2 (BL2) labs, and during spill cleanup.

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PPE Type	Requirement
Respiratory	Work with phenol-containing solutions must be done in a fume hood. If work with phenol is
Protection	conducted without engineering controls, such as on an open bench or in an un-ducted
	biosafety cabinet, contact EHS for an assessment.
	Other common kit components do not require respiratory protection.

Before Starting Work

- Ensure that a written experimental protocol including safety information is available.
- Review the manufacturer's SDS and <u>Safe Chemical Work Practices</u>.
- Review Sections 7 (Handling & Storage) and 10 (Stability & Reactivity) of the SDS for incompatible chemicals.
- Be familiar with the general University emergency procedures in the <u>Lab Emergency Response Guide</u>.
- Identify the location of the nearest eyewash and shower and verify that they are accessible.
- Locate and verify that appropriate spill cleanup materials are available.

During Work

- Avoid any contact with kit components! Wear PPE as outlined in the PPE section of this document.
- Wash hands and forearms thoroughly with soap and water each time gloves are removed.
- Be aware of potential incompatibilities, such as with bleach (see <u>Appendix 1: Bleach Incompatibility</u>).
- Keep all containers tightly closed when not in use and during transport.
- If using phenol-containing products like Qiazol, work with an exposure control device such as a chemical fume hood and wear a lab coat, double nitrile gloves, and eye protection.

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After Completing Work

- Clean work area.
- Since biological materials are inactivated by cell-lysis reagents in nucleic acid kits, there is no need to also
 decontaminate them with bleach. This kind of chemical-containing biological waste should be disposed of
 as a chemical waste. Refer to Appendix 2: Qiagen Kit Components for detailed information on disposal of
 common Qiagen kit components.
- For Qiagen materials not listed, or for waste from other manufacturer's kits, dispose of waste following standard hazardous waste procedures.
- Return kit components to the kit box provided by the manufacturer. If not available, place each
 component in appropriate storage following the <u>Lab Chemical Storage Guide</u> while maintaining separation
 between incompatible kit components.
- Store kit components away from incompatible materials, including but not limited to bleach.
- Wash hands and forearms thoroughly with soap and water before leaving the lab.

Emergency Procedures

Refer to the <u>Lab Emergency Response Guide</u> and the information in this section.

Notify the Principal Investigator (PI) or supervisor of any exposures or incidents involving nucleic acid extraction kits. The PI or their designee must <u>report all exposures or injuries</u> within 24 hours.

First Aid

- For exposures involving phenol-containing solutions, such as Qiazol or Trizol, refer to <u>Lab Safety</u>
 Guideline: Phenol.
- For serious medical emergencies, go to the closest emergency room or call 911.

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• For non-emergency medical attention, contact the Exposure Response Call Center (ERCC) hotline by calling 1-866-360-8100.

Skin Contact

Treatment starts immediately following exposure. If exposure involves phenol-containing solutions, refer to Lab Safety Guideline: Phenol.

- Remove all potentially contaminated clothing and jewelry.
- Flush affected skin area using sink if on hands or arms or safety shower for 15 minutes.
- Follow up with the ERCC by calling 1-866-360-8100.

Eye Contact

- Rinse eyes at an eyewash station for at least 15 minutes.
- Seek medical attention.

Inhalation

- Move person to a location with fresh air.
- Seek medical attention.

Ingestion

- Do not induce vomiting if kit components are swallowed.
- Never give anything by mouth to an unconscious person.
- Call 911 for medical assistance.

Sharps Injury

- Immediately wash the area with soap and water for at least 15 minutes.
- Seek medical assistance.

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Spill or Chemical Reaction Response

For spills involving phenol-containing solutions, such as Qiazol or Trizol, refer to <u>Lab Safety Guideline</u>: Phenol.

For spills not involving phenol-containing solutions:

- Alert others of the spill.
- If liquid is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, after cleaning with detergent and water clean with a 10% bleach solution.
- If a kit has reacted or a spill cannot be contained, contact:
 - Harvard Medical School (HMS) or Harvard School of Dental Medicine (HSDM) labs: Call 617-432-1901.
 - Other labs: Call the Operations Center at 617-495-5560.
- Remain in a safe location until EHS or other response personnel arrive.

Many kit components are incompatible with bleach. If inadvertently mixed with bleach, a noticeable chemical reaction will occur. In that case, do the following:

- Move container to fume hood if safe to do so.
 - Keep the container un-capped to prevent container over-pressurization.
 - Close sash. Activate the hood's emergency purge mode if available.
 - Place sign on fume hood indicating not to use until reaction has cooled.
 - Notify the Lab Safety Officer and PI of the incident.
 - The following day, if bottle is cool and there are no indications of a continuing reaction, replace the cap
 or transfer to a bottle with a lid, then send out as hazardous chemical waste. Include the chemicals in
 the kit components and bleach on the waste tag.
- If not safe to move the container to a fume hood, evacuate the room and contact:

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HMS or HSDM labs: Call 617-432-1901.

• Other labs: Call the Operations Center at 617-495-5560.

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Appendix 1: Bleach Incompatibility

This table lists the most common chemicals in nucleic acid kits that may react with bleach.

Incompatible Chemicals and Agents	Possible Results of Mixing with Bleach
Acetic Acid	Release of toxic chlorine gas (reaction/release
	may occur violently)
Guanidine Salts, such as Guanidine Hydrochloride and	Release of toxic gases which can include
Guanidine Thiocyanate, found in many lysis buffers	chloramines, chlorine, and hydrogen cyanide
Isopropanol	Release of chloroform and other potentially
	harmful biproducts
Phenol	Release of toxic gases, such as chlorine and
	chlorinated phenolic compounds
Sodium Perchlorate	Exothermic reaction

Common Kit Information

The following commonly used Qiagen kits have known bleach reactions. This is not an exhaustive list of all kits that react with bleach.

- QIAprep Miniprep.
- BioSprint PCR Purification.
- QIAquick Multiwell PCR Purification (8 and 96).
- QIAamp Virus BioRobot 9604.
- QIAquick.
- RNeasy Plus.

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Appendix 2: Qiagen Kit Components

The information in this table only applies to Qiagen reagents and kits when they are not mixed with any other chemicals. For kits by other manufacturers, refer to their product SDSs for information. Refer to Section 3 of the SDS for a list of hazardous components. Refer to Section 7, Handling & Storage, and Section 10, Stability & Reactivity, for information on incompatible chemicals.

Please contact your <u>Lab Safety Advisor</u> for advice on proper management and disposal of any Qiagen reagents not listed on this table, or for reagents from other manufacturers.

Reagent name	Hazardous chemical components (non-hazardous components make up the balance)	Manage as hazardous waste?	Sink disposal?	Known bleach reactions
Buffer AE	None	No	Yes (within daily maximum volume) ¹	
Buffer AL	Guanidinium Chloride, 25-50%	No	Yes	Reacts with bleach
Buffer ALO	Sodium Dodecyl Sulphate, 0.1- 1.0%	No	Yes	
Buffer AP1	Sodium Dodecyl Sulphate, 0.1- 1.0%	No	Yes	

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¹ Can be disposed of down the drain, but the maximum combined total volume that can be discharged is 100 grams of solute per lab per day. After drain disposal, please flush with at least 10-20-fold excess of water to thoroughly rinse out the sink and sink trap.



Hazardous chemical components	Manage as	Sink disposal?	Known
(non-hazardous components	hazardous		bleach
make up the balance)	waste?		reactions
Acetic Acid, 10-25%	Yes		
Guanidinium chloride, 50-100%	No	Yes	Reacts with
			bleach
Zinc Chloride, 1.0-2.5%	Yes		
Sodium Dodecyl Sulphate, 2.5-	No	Yes	
10%			
None	No	Yes (within daily	
		maximum	
		volume) ^{Error! Bookmark}	
		not defined.	
Guanidinium Chloride, 50-100%	No	Yes	Reacts with
			bleach
Sodium Azide, 0.1%	Yes		
Cetrimonium Bromide, 0.1-1.0%	Yes		
None	No	Yes (within daily	
		maximum	
		volume) ^{Error! Bookmark}	
		not defined.	
	(non-hazardous components make up the balance) Acetic Acid, 10-25% Guanidinium chloride, 50-100% Zinc Chloride, 1.0-2.5% Sodium Dodecyl Sulphate, 2.5-10% None Guanidinium Chloride, 50-100% Sodium Azide, 0.1% Cetrimonium Bromide, 0.1-1.0%	(non-hazardous components make up the balance)hazardous waste?Acetic Acid, 10-25%YesGuanidinium chloride, 50-100%NoZinc Chloride, 1.0-2.5%YesSodium Dodecyl Sulphate, 2.5- 10%NoNoneNoGuanidinium Chloride, 50-100%NoSodium Azide, 0.1%YesCetrimonium Bromide, 0.1-1.0%Yes	(non-hazardous components make up the balance) hazardous waste? Acetic Acid, 10-25% Yes Guanidinium chloride, 50-100% No Yes Zinc Chloride, 1.0-2.5% Yes Sodium Dodecyl Sulphate, 2.5-10% No Yes (within daily maximum volume) Errorl Bookmark not defined. Guanidinium Chloride, 50-100% No Yes Sodium Azide, 0.1% Yes Cetrimonium Bromide, 0.1-1.0% Yes None No Yes (within daily maximum volume) Errorl Bookmark volume) Errorl Bookmark

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Reagent name	Hazardous chemical components (non-hazardous components make up the balance)	Manage as hazardous waste?	Sink disposal?	Known bleach reactions
Buffer EC	None	No	Yes (within daily maximum volume) ^{Error! Bookmark} not defined.	
Buffer ETR	None	No	Yes (within daily maximum volume) ^{Error! Bookmark} not defined.	
Buffer EX Reaction Buffer	None	No	Yes (within daily maximum volume) ^{Error! Bookmark} not defined.	
Buffer N3	Guanidinium Chloride, 25-50% Acetic Acid, 10-25%	Yes		Reacts with bleach
Buffer P1	None	No	Yes (within daily maximum volume) Error! Bookmark not defined.	
Buffer P2	Sodium Dodecyl Sulphate, 0.1- 1.0%	No	Yes	

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Reagent name	Hazardous chemical components	Manage as	Sink disposal?	Known
	(non-hazardous components	hazardous		bleach
	make up the balance)	waste?		reactions
Buffer P3	Acetic Acid, 10-25%	Yes		
Buffer PE	None	No	Yes (within daily	
			maximum	
			volume) ^{Error! Bookmark}	
			not defined.	
Buffer PB	Guanidinium Chloride, 25-50%	Yes		Reacts with
	Isopropanol, 25-50%			bleach
Buffer PNI	Guanidinium Chloride, 25-50%	Yes		Reacts with
	Isopropanol, 25-50%			bleach
Buffer PM	Guanidinium Hydrochloride, 25-	No	Yes	Reacts with
	50%			bleach
Buffer QBT	Isopropanol, 10-25%	Yes		Reacts with
				bleach
Buffer QC	Isopropanol, 10-25%	Yes		Reacts with
				bleach
Buffer QF	Isopropanol, 10-25%	Yes		Reacts with
				bleach
Buffer QG	Guanidinium Chloride, 50-100%	No	Yes	Reacts with
				bleach

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Reagent name	Hazardous chemical components	Manage as	Sink disposal?	Known
	(non-hazardous components	hazardous		bleach
	make up the balance)	waste?		reactions
Buffer QLE	None	No	Yes (within daily maximum volume) Error! Bookmark not defined.	
Buffer QLL	Dimethyl-N-Lauryl-N- (3Sulfopropyl)-Ammonium-Betain, 2.5-10% Guanidinium Chloride, 1.0-2.5% Guanidine Thiocyanate at 1.0- 2.5%	Yes		Reacts with bleach
Buffer QLW	None	No	Yes (within daily maximum volume) ^{Error! Bookmark} not defined.	
Buffer QN	Isopropanol, 10-25%	Yes		Reacts with bleach
Buffer QS	Isopropanol, 10-25%	Yes		Reacts with bleach
Buffer QXI	Sodium Perchlorate, 50-100%	Yes		Reacts with bleach

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Reagent name	Hazardous chemical components (non-hazardous components	Manage as hazardous	Sink disposal?	Known bleach
	make up the balance)	waste?		reactions
Buffer RDD	None	No	Yes (within daily maximum volume) ^{Error! Bookmark} not defined.	
Buffer RNAlater	None	No	Yes (within daily maximum volume) Error! Bookmark not defined.	
Buffer RLC	Guanidinium Chloride, 50-100%	No	Yes	Reacts with bleach
Buffer RLT	Guanidinium Thiocyanate, 25-50%	No	Yes	Reacts with bleach
Buffer RLT Plus	t- Octylphenoxypolyethoxyethanol, 1.0-10%	No	Yes	Reacts with bleach
Buffer RPE (concentrate)	None	No	Yes (within daily maximum volume) Frror! Bookmark not defined.	

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Reagent name	Hazardous chemical components (non-hazardous components	Manage as	Sink disposal?	Known bleach
	make up the balance)	waste?		reactions
Buffer RT	Trometamol, 2.5-10%	No	Yes	
	Hydrogen Chloride, 2.5-10%			
Buffer RW1	Guanidine Thiocyanate, 2.5-10%	No	Yes	Reacts with
	Ethanol, 2.5-10%			bleach
Buffer S3	Acetic Acid, 2.5-10%	No	Yes	
dNTP Mix	None	No	Yes	
Effectene	None	No	Yes	
Transfection				
Buffer				
Enhancer	None	No	Yes	
Exonuclease	None	No	Yes (within daily	
Solvent (Buffer)			maximum	
			volume) ^{Error! Bookmark}	
			not defined.	
gDNA Wipeout	Trometamol, 2.5-10%	No	Yes	
Buffer				
GelPilot Loading	Trometamol, 2.5-10%	No	Yes	
Dye				

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Reagent name	Hazardous chemical components	Manage as	Sink disposal?	Known
	(non-hazardous components	hazardous		bleach
	make up the balance)	waste?		reactions
LyseBlue	None	No	Yes	
Lysozyme	None	No	Yes	
Multiplex PCR	None	No	Yes	
Master Mix 2x				
Omniscript	None	No	Yes (within daily	
Reverse			maximum	
Transcriptase			volume) ^{Error! Bookmark}	
			not defined.	
OneStep Enzyme	None	No	Yes (within daily	
Mix			maximum	
			volume) ^{Error! Bookmark}	
			not defined.	
OneStep RT-PCR	Trometamol, 2.5-10%	No	Yes	
Buffer				
pH Indicator I	Cresol Red, <1%	No	Yes	
Proteinase K	Proteinase, <1%	Yes		

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Reagent name	Hazardous chemical components	Manage as	Sink disposal?	Known
	(non-hazardous components	hazardous		bleach
	make up the balance)	waste?		reactions
Quagen Resin	None	No	Yes (within daily maximum volume) ^{Error! Bookmark} not defined.	
QIAEX II	Sodium Perchlorate, 50-100%	Yes		Reacts with
Suspension				bleach
QIAzol Lysis	Phenol, 25-50%	Yes		Reacts with
Reagent	Guanidine Thiocyanate, 10-25%			bleach
Q-Solution 5x	None	No	Yes	
Quantifast SYBR	1,2,4-Triazole, 1.0-2.5%	No	Yes	Reacts with
Green PCR Master	2amino/2/(hydroxymethyl)propan			bleach
Mix	e-1,3 diolhydrochloride, 1.0-2.5%			
QuantiScript	None	No	Yes (within daily	
Reverse			maximum	
Transcriptase			volume) ^{Error! Bookmark}	
QuantiScript RT	None	No	Yes (within daily	
Buffer 5x			maximum volume) ^{Error! Bookmark} not defined.	

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Reagent name	Hazardous chemical components	Manage as	Sink disposal?	Known
	(non-hazardous components	hazardous		bleach
	make up the balance)	waste?		reactions
QuantiTect RT Mix	None	No	Yes (within daily maximum volume) ^{Error! Bookmark} not defined.	
RNase A Solution	Ribonuclease, 2.5-10%	No	Yes	
RT Primer Mix	None	No	Yes	
SuperFect Transfection Reagent	None	No	Yes	
SYBR Green RT- PCR Master Mix	Sodium Azide, 0.27% Tris Buffer, 99.23%	Yes		

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