

Sodium Hypochlorite (Bleach) Safety Fact sheet Warning: May Not Play Well with DNA/RNA Kits

Sodium hypochlorite, the active ingredient in chlorine bleach, is routinely used in the laboratory to decontaminate surfaces and equipment or deactivate biological materials by inactivating vegetative bacteria, fungi, lipid and non-lipid viruses, and other liquid specimens. Bleach is very reactive and if it mixes with incompatible chemicals, it can produce high hazard by-products and toxic gases.

DNA/RNA Kit Incompatible Warning: Some trademarked reagents and "kits" used in the lab may contain hazardous materials and/or ingredients that are incompatible with bleach. Many chemicals in these kits already contain a deactivating agent.

Do not use bleach with these kits!

Kit constituents that are incompatible with bleach	Possible reactions and symptoms
Alcohols e.g., ethanol, methanol, isopropanol, propanol (may be found in buffers of binding beads such as QIAGEN kits and Blood & Cell culture kits)	Forms chloroform, hydrochloric acid, chloroacetone or dichloroacetone. Low levels of chloroform exposure could result in fatigue, dizziness, and headache. Elevated levels of chloroform may damage the liver and kidneys.
Guanidine Salts e.g., guanidine hydrochloride, guanidinium chloride, guanidine thiocyanate, guanidine isothiocyanate (found in lysis and wash buffers, which also contains phenol. Products include Qiagen kits, lysis buffers, and TRIzol® RNA Purification Kits)	Forms toxic gases (e.g., chloramine, chlorine, and hydrogen cyanide) and can form highly reactive compounds. Low levels of exposure may result in eye and oral mucous membrane irritation, dizziness and nausea - while exposure to high levels may be fatal.

Always review the Safety Data Sheet (SDS) and the manufacturer's guidelines to determine the chemical compatibility of chemicals or proprietary material with the deactivating agent.

What are the hazards of Bleach?

 Bleach is an oxidizer and corrosive. Exposures may cause irritation or damage to the skin, eyes, and the respiratory tract. Vapors can cause serious discomfort or even acute distress requiring medical attention.



• Bleach is incompatible with many chemicals found in the laboratory and DNA/RNA kit components. Mixing bleach with incompatible chemicals can produce toxic gases that can potentially damage the eyes, skin, lungs, vocal cords, nervous system, liver, and kidneys.

Bleach Incompatibilities

The following list is not exhaustive and includes commonly encountered chemicals used in the laboratory.

Bleach-incompatible chemicals and materials	Possible reactions and symptoms from mixing with bleach
Acids and acidic compounds e.g., hydrochloric acid, sulfuric acid, hydrofluoric acid, phosphoric acid, aluminum chloride	Forms toxic chlorine gas. Low levels of exposure may result in eye and oral mucous membrane irritation, dizziness, and nausea - while exposure to high levels may be fatal.
Alcohols e.g., ethanol, methanol, isopropanol, and propanol	Forms chloroform, hydrochloric acid, chloroacetone or dichloroacetone. Low levels of chloroform exposure could result in fatigue, dizziness, and headache. Elevated levels of chloroform may damage the liver and kidneys.
Ammonia-containing compounds e.g., Ammonium chloride, ammonium sulfate, quaternary ammonium salts	Forms toxic chlorine and chloramine gases. Low levels of exposure may result in eye and oral mucous membrane irritation, dizziness, and nausea - while exposure to high levels may be fatal.
Guanidine Salts e.g., Guanidine hydrochloride, guanidinium chloride, guanidine thiocyanate	Forms toxic gases (e.g., chloramine, chlorine, and hydrogen cyanide) and can form highly reactive compounds. Low levels of exposure may result in eye and oral mucous membrane irritation, dizziness, and nausea - while exposure to high levels may be fatal.
Metals Stainless steel, iron, copper, nickel	Mixing with bleach releases oxygen in a closed system (e.g., piping, equipment) which can lead to pressure build-up and rupture. Bleach will corrode metal including metal wastewater pipes.
Organic chemicals e.g., Organic solvents and polymers, amines, ethylene glycol, formic acid, insecticides, fuels and fuel oils	Forms chlorine gas, and chlorinated organics which are toxic and/or carcinogenic. Low levels of exposure may result in eye and oral mucous membrane irritation, dizziness, and nausea - while exposure to high levels may be fatal.
Reducing agents e.g., Sodium bisulfite, sodium hydrosulfate, sodium sulfate	Boiling or splashing hazard if mixed with bleach.



Safe Work Practices

Proper concentrations

For disinfection of most biologicals (Exception: prions and prion-like proteins), after dilution, working bleach solutions must contain between 0.5% and 2% sodium hypochlorite to be an effective disinfectant. Hypochlorite concentration in household bleach varies by manufacturer. Many household bleach solutions contain 5.25% sodium hypochlorite, and a 1:10 dilution (5250 ppm Cl) will produce a 0.53% hypochlorite solution. Use of bleach solutions with lower hypochlorite concentrations will not provide the proper level of disinfection.

→ Bleach is not stable at dilute concentration. Users should prepare a fresh bleach solution regularly. Prepare a fresh working dilution of sodium hypochlorite weekly and indicate the preparation date on the bottle.

Chlorine (hypochlorite) compounds are effective in inactivating vegetative bacteria, fungi, lipid and non-lipid viruses, *Coxiella burnetii* and Tuberculosis. Chlorine compounds have some effect in inactivating bacterial spores:

- Recommended working dilution: 5250 ppm (1:10 dilution of household bleach of 5.25% sodium hypochlorite)
- Recommended for floors, spills (inactivating liquid specimens), bench tops and contaminated clothing. Do not use bleach on electronic equipment, optical equipment or unpainted stainless steel
- Undiluted bleach and other disinfectants must not go down the drain or be mixed with other materials
 - Only 1:10 dilutions of bleach that have been mixed with adequate levels of protein (such as those found in tissue culture media containing fetal bovine serum) can be poured down the drain
 - Undiluted bleach is substantially more reactive than diluted bleach, and has even been reported to generate toxic gases such as cyanogen and chloramine when mixed with Luria broth in a ~1:1 ratio

Refer to the <u>Biosafety Manual</u> for additional information and guidance on selecting appropriate disinfectants, or for appropriate bleach use with prions and prion-like proteins.

How can I protect myself?

Engineering Controls

- Use stock or working bleach solutions in a well ventilated area
- Work in a certified chemical fume hood when using volumes greater than 1000mL

¹ Stanford University Biosafety Manual



Work Practices

- Purchase and use the lowest volume and concentration necessary
- Do not use bleach in diluted concentrations greater than 10% unless working with prions
- Avoid contact with eyes, skin, and clothing
- Verify the SDS and manufacturer's guidelines for chemical compatibility before mixing bleach
- Never mix bleach with incompatible chemicals, an unknown chemical, or mixture
- Do not autoclave bleach solutions
- Tightly recap bleach bottle and store in a corrosive-resistant container. Store below eye level with compatible chemicals (Stanford Compatible Storage Group E).
- Do not store bleach on the floor. Store in secondary containment

Personal Protective Equipment (PPE)

- Wear appropriate PPE, which minimally includes: safety goggles, nitrile gloves, lab coat, long pants or equivalent, and closed-toe shoes
- Additional PPE when working with larger volumes may include face shields and impervious apron/sleeves
- Consult the glove manufacturer's chemical resistance data when selecting gloves effective for chemicals in use

Drain Disposal and Waste Management

- 1:10 diluted bleach that has reacted with proteins such as tissue culture or blood may be drain disposed
- See the <u>Biohazardous Waste Poster</u> for information on disposal of biohazardous waste
- Never pour unused, undiluted or dilutions higher than 1:10 bleach solutions down the drain. Create a hazardous waste tag and request a pickup (http://wastetag.stanford.edu)
- Attach a waste tag and collect waste separately so that bleach or acidic solutions are not accidentally added to sample preparation kit waste
- Follow Toxin Inactivation guidance for Select Agents and Toxins
- Visit Water Planning and Stewardship Website for information on Stanford's <u>Wastewater Best</u> <u>Management Practices</u>

Emergency procedures

Laboratories should clean up small spills themselves, provided they are knowledgeable of the hazards and have the proper PPE. For information on cleaning up spills, see the <u>Spill Response Standard Operating Procedure (SOP)</u>. For larger spills, call EH&S at 650-725-9999. For health emergencies, call 911 (9-911 from a campus phone).

References

- Bretherick's handbook of reactive chemical hazards
- Stanford University <u>Biosafety Manual</u>
- Stanford University Handling Mixed Waste
- https://www.ncbi.nlm.nih.gov/books/NBK537213/
- https://www.atsdr.cdc.gov/phs/phs.asp?id=51&tid=16
- Norval, G.A. "Chronic Lung Impact on Laboratory Worker Exposed to Chloramines and Cyanogen Chloride," ACS Chem. Health Saf., 27(2), 2020, p. 129-132.