Disposal of Electrophoresis Buffer Solutions and Gels

Overview: Electrophoresis gels are commonly used in molecular biology laboratories for the identification of DNA and proteins. These gels will typically be agarose-based or polyacrylamide-based. This electrophoresis process utilizes an organic fluorescence dye or an inorganic stain such as Silver (which is an EPA regulated material) to stain the nucleic acids or proteins. Waste by-products of the DNA identification process must be managed and disposed in a manner to protect public health and the environment.

Purpose: To ensure safe, prudent disposal as well as reduce the amount of Hazardous Waste material generated at the University of Rochester. This can be accomplished by choosing less toxic materials and work practices that minimize the overall quantity of waste generated as well as the toxicity of the waste material itself. In cases where safer materials or work practices cannot be employed, waste collection methods per University and regulatory agency requirements are to be followed.

Background: There are a number of different protocols and dyes used in the preparation and use of electrophoresis gels. Gels can be cast with or without dyes. The nucleic acids/proteins can be stained by adding the dye to the sample before electrophoresis, the dye can be added to the running buffer before electrophoresis, or the gel can be placed in a dye solution after electrophoresis has been completed.

Waste Management: Waste disposal requirements will vary depending on the dye used and the methodology used to stain the cells.

Silver containing waste is regulated as a Hazardous Waste by the USEPA. Drain disposal is also forbidden by the Monroe County Sewer District. All unwanted stock solutions gels, contaminated debris (gloves, paper towels, pipet tips) and running buffer solutions that contain silver must be collected for disposal by the Hazardous Waste Management Unit (HWMU).

MUTAGENIC DYES

Ethidium Bromide, Propidium Iodide, Acridine Orange, SYBR® Green I, SYBR® Green II, SYBR® Gold, GelStar. These dyes have been determined to have mutagenic properties.

All gels that have been cast with these dyes in them, unwanted dye stock solutions, and all contaminated debris must be collected for disposal by the HWMU.

Gels that have undergone electrophoresis and staining, and then have been destained - where all excess dye has been washed out the gel (the only dye left in the gel is a trace amount contained in the nucleic acid/protein sample material) can be discarded in the trash.

Contaminated “non-sharp” lab debris (e.g., gloves, pads, towels, tubes, etc.) should be collected and disposed of through the HWMU.

The spent running buffer solutions and destaining solutions that contain the dyes can either be collected and disposed of through the HWMU or collected and run through an approved

filter device. The buffer solutions that have been run through the approved filter should be checked under an appropriate light source for complete removal of the dyes, and if it passes (does not fluoresce), the liquid can be disposed of down the drain with a copious amount of water as long as it contains no other materials that would cause it to be regulated as a Hazardous Waste.

The filters that have been used up and are no longer effective must be disposed of through the HWMU.

NONMUTAGENIC DYES

SYBR® Safe, GelRed, **GelGreen**, and **EvaGreen**. These dyes have been determined to be nonmutagenic in Ames testing by independent licensed testing laboratories.

All gels and contaminated "non-sharp" lab debris (e.g., gloves, pads, towels, tubes, etc.) that are processed using this dye can be discarded in the trash.

Spent running buffer solutions and destaining solutions that contain the dyes can either be collected and disposed of through the HWMU or collected and run through an approved filter device.

The buffer solutions that have been run through the approved filter should be checked under the appropriate light source for complete removal of the dyes, and if it passes (does not fluoresce), the liquid can be disposed of down the drain with a copious amount of water as long as no other materials are present that would cause the material to be a Hazardous Waste.

The filters that have been used up and are no longer effective must be disposed of through the HWMU.

Waste Management Procedures for Collection of Materials for Disposal through the Hazardous Waste Management Unit

Mutagenic or Toxic Electrophoresis Gels and Contaminated "Non-Sharp" Lab Debris

1. Collect electrophoresis gels and contaminated "non-sharp" lab debris (e.g., gloves, pads, towels, tubes, etc.) into a plastic container (suitable for holding chemicals), or 5 gallon bucket (depending on the volume of waste generated). This container should have a plastic bag as an inner liner. The container must remain closed at all times except when immediately adding or removing wastes from the container. Contact the Hazardous Waste Management Unit if you need a 5 gallon bucket to collect your waste.
2. Mark on the container's label which waste constituents are present in the pail (e.g., "Hazardous Waste - Ethidium Bromide Contaminated Gels, Gloves, Paper").
3. **NO SHARPS**: No sharp items (e.g., needles, Pasteur pipettes, razor blades, etc) are to be placed into the containers or 5-gallon pails. See below for the proper means for disposing of contaminated sharps lab debris.
4. Disposal: Once the 5-gallon pail is 75% full, fill out a Hazardous Waste tag and call x5-2056 for a pickup. An empty replacement pail will be provided at the time of the collection if needed.

Collection and Disposal of Chemically Contaminated Sharps

1. Chemically contaminated sharps (needles, Pasteur pipettes, razor blades) must be collected in an approved sharps shelter (**NOT RED** – use the white/translucent or yellow ones). It must be labeled "Hazardous Waste – Chemically Contaminated Sharps". Any biohazard labels should be removed or completely defaced. When the shelter is full, fill out a Hazardous Waste Tag and call x5-2056 for a pickup.

Summary

Disposal of Waste Products From Gel Electrophoresis Using Dyes With Mutagenic Properties	
Name of Dye Used	**Disposal Instructions**
MUTAGENIC DYES Ethidium Bromide Propidium Iodide Acridine Orange SYBR Green I SYBR Green II SYBR Gold GelStar	All gels that have been cast with these dyes in them and unwanted dye stock solutions should be collected and disposed of through the HWMU. Gels that have undergone electrophoresis and staining, and then have been destained - where all excess dye has been washed out the gel (the only dye left in the gel is a trace amount contained in the nucleic acid/protein sample material) can be discarded in the trash. Spent running buffer solutions and destaining solutions that contain the dyes can either be collected and disposed of through the HWMU or collected and run through an approved filter device. The filters that have been used up and are no longer effective must be disposed of through the HWMU. Contaminated "non-sharp" lab debris (e.g., gloves, pads, towels, tubes, etc.) should be collected and disposed of through the HWMU. Chemically contaminated sharps (needles, Pasteur pipets, razor blades) must be collected in an approved sharps shelter (**NOT RED** – use the white/translucent or yellow ones). It must be labeled "Hazardous Waste – Chemically Contaminated Sharps".

Disposal of Waste Products From Gel Electrophoresis Using Non-mutagenic Dyes	
Name of Dye Used	**Disposal Instructions**
NONMUTAGENIC DYES SYBR Safe GelGreen GelRed EvaGreen	All gels and contaminated "non-sharp" lab debris (e.g., gloves, pads, towels, tubes, etc.) that are processed using this dye can be discarded in the trash. Unwanted dye stock solutions, spent running buffer solutions and destaining solutions that contain the dyes can either be collected and disposed of through the HWMU or collected and run through an approved filter device. The filters that have been used up and are no longer effective must be disposed of through the HWMU.

	Chemically contaminated sharps (needles, Pasteur pipets, razor blades) must be collected in an approved sharps shelter (**NOT RED** – use the white/translucent or yellow ones). It must be labeled "Hazardous Waste – Chemically Contaminated Sharps".

Approved Filters for Electrophoresis Dye Solutions

Company Name	Product Name	Product Code	Phone Number	Website
Amresco, Inc.	Destaining Bags	E732	800-829-2805	http://www.amresco-inc.com/
BD Biosciences-Clontech	BondEX Detoxicification Cartridges	K3080-1	877-232-8995	http://www.clontech.com/
VWR International	Extractor Waste Reduction System	28165-500	800-932-5000	http://www.vwrsp.com/

References:

Ethidium Bromide MSDS: http://msds.ehs.cornell.edu/msds/siri/files/cfg/cfggr.html
Propidium Iodide MSDS: http://msds.ehs.cornell.edu/msds/siri/files/cfg/cfglw.html
Acridine Orange MSDS: http://www.immunochemistry.com/MSDS.htm
SYBR® Safe Ames Testing: http://probes.invitrogen.com/media/publications/494.pdf, http://probes.invitrogen.com/media/pis/mp33100.pdf
SYBR® Green I: http://probes.invitrogen.com/media/pis/mp07567.pdf
SYBR® Green II: http://probes.invitrogen.com/media/pis/mp07568.pdf
SYBR® Gold: http://probes.invitrogen.com/media/pis/mp11494.pdf
GelRed, GelGreen Safety Report**:**
http://www.biotium.com/product/product_info/Newproduct/GelStains.asp
EvaGreen Safety Report:
http://www.biotium.com/product/price_and_info.asp?item=31000&Sub_Section=09A
GelStar Product Protocol:
http://www.cambrex.com/Content/Documents/Bioscience/GelStar%2818111%29.pdf
GelRed, GelGreen Ames Testing:
www.biotium.com/product/product_info/Safety_Report/GR%20&%20GG%20safety.pdf
EvaGreen Ames Testing:
www.biotium.com/product/product_info/Safety_Report/EG%20safety.pdf