Lab Standard Operating Procedure:

**Buffer-Gradient Polyacrylamide Gel Preparation**a

PI: Room & Building:

Department: Research Group:

Date: Pertains to Lab Protocol:

**PROCEDURE**

Attach the experimental protocol for the preparation of buffer-gradient polyacrylamide gel.

**POTENTIAL HAZARDS**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Principal Materials Used | Corrosive | Irritant | Sensitizer | Reproductive toxin | Acutely Toxic | Carcinogen | Flammable | Combustible | Water-Reactive | Shock-Sensitive | Pyrophoric | Oxidizer | Biotoxin | Other Comments: |
| Acrylamide |  | X |  | X | X | X | X |  |  |  |  |  |  | See below **1.** |
| N,N’-methylenebisacrylamide |  | X |  |  |  |  |  |  |  |  |  |  |  | See below **2.** |
| TEMED (N,N,N’,N’-  tetramethylethylenediamine) | X |  |  |  |  |  |  | X |  |  |  |  |  |  |
| 5X TBE |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Tris base |  | X |  |  |  |  |  |  |  |  |  |  |  |  |
| Boric acid |  | X |  |  |  |  |  |  |  |  |  |  |  | See below **3.** |
| 0.5M EDTA (pH 8.0) |  | X |  |  |  |  |  |  |  |  |  |  |  |  |
| Ammonium persulfate | X | X |  |  |  |  |  |  |  |  |  | X |  |  |
| Urea (ultrapure) |  | X |  |  |  |  |  |  |  |  |  |  |  |  |
| Sucrose |  | X |  |  |  |  |  |  |  |  |  |  |  |  |
| Bromophenol blue |  | X |  |  |  |  |  |  |  |  |  |  |  |  |
| Potassium hydroxide | X | X |  |  | X |  |  |  |  |  |  |  |  | See below **4.** |
| Methanol |  | X |  |  |  |  | X |  |  |  |  |  |  |  |

**1.** Acrylamide is a potent neurotoxin and is absorbed through the skin. The effects of acrylamide are cumulative. Wear gloves and a mask when weighing powdered acrylamide. Although polyacrylamide is considered to be nontoxic, it should be handled with care because of possibility that it might contain small quantities of unpolymerized acrylamide (e.g. wear gloves and a mask when washing the pipette and the syringe). Periodically treat area where unpolymerized acrylamide is used with 1.6% potassium persulfate, then with 1.6% sodium metabisulfite. Let stand for 30 minutes, and then wash with plenty of water.

**2.** May affect central nervous system. Wear gloves and a mask when weighing powdered N,N’-methylenebidacrylamide.

**3.** May affect central nervous system, liver, and kidneys.

**4.** Highly corrosive. Causes severe burns to skin, eyes, respiratory tract, and gastrointestinal tract. Extremely destructive to all body tissues.

**ENGINEERING/VENTILATION CONTROLS**

* Acrylamide, methylenebisacrylamide, and ammonium persulfate should be handled in a laboratory hood. No open bench work.
* Cover the working area with plastic-backed protective paper because it is almost impossible to pour the sequencing gels without dripping acrylamide solutions onto the bench.

**REQUIRED PERSONAL PROTECTIVE EQUIPMENT**

(Refer to your lab’s PPE Assessment Report, supplemented with information here)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Protective Clothing | Nitrile | PVC (Vinyl) | PVA | Latex/Natural rubber | Neoprene | Butyl | Additional Specifications |
| Aprons/Lab coat/Smock |  |  |  |  |  |  |  PE, PVC   Avoid traditional cotton-polyester white lab coat |
| Shoe Cover |  |  |  |  |  |  |  Impervious boots or PVC disposable shoe  coverings are recommended. |
| Full Body Cover |  |  |  |  |  |  |  |
| Head Cover |  |  |  |  |  |  |  |
| Gloves/Gauntlets |  |  |  |  |  |  |  Gloves should be worn when preparing and handling the gel solutions.   Gloves are for splash protection; not for immersion protection.   Double-gloved is recommended given the  diversity of chemical hazards and highly corrosive chemicals. It is recommended that two different glove types and colors be used for best protection.   Immediately replace with new gloves when splash occurs. |
| Acrylamide | X | X |  |  | X | X |
| N,N’-methylenebisacrylamide |  | X |  | X |  | X |
| TEMED | X |  |  |  |  |  |
| Boric acid | X |  |  |  | X | X |
| 0.5M EDTA (pH 8.0) |  |  |  | X |  |  |
| Ammonium persulfate |  |  |  | X | X |  |
| Urea (ultrapure) | X |  |  | X | X |  |
| Bromophenol blue |  |  |  | X |  |  |
| Potassium hydroxide | X |  |  |  | X | X |
| Methanol |  |  |  |  |  | X |

**SAFETY REFERENCES**

Additional chemical safety information, including MSDSs and other information, is available electronically as tools at [ehs.harvard.edu/programs/safe-chemical-work-practices](http://ehs.harvard.edu/programs/safe-chemical-work-practices).

**WASTE DISPOSAL**

Refer to the *Laboratory Waste Guide* posted at [ehs.harvard.edu/node/7699](http://ehs.harvard.edu/node/7699).

**EMERGENCY PROCEDURES**

(Refer to the [Emergency Response Guide](http://www.ehs.harvard.edu/programs/emergency-guidance) posted in your lab, supplemented with information here)

**Fire/Evacuation**: 0.5M EDTA, pH 8.0 may produce toxic fumes under fire conditions. Do not use water as extinguishing medium for ammonium persulfate.

***Reference:***

a. J. Sambrook, E. F. Fritsch, T. Maniatis, “Denaturing Polyacrylamide Gels,” *Molecular Cloning: A Laboratory Manual, 2nd ed.,* Cold Spring Harbor Laboratory Press, 1989, p13.45-53.