**HEPES-Sucrose Cutting Solution**

1. **Scope and Applicability:** Method for preparing HEPES-Sucrose Cutting Solution, used by the Molecular Genetics team for animal perfusion following anesthesia.
2. **Materials:**
   1. NaCl (Sigma S5886 or equivalent)
   2. HEPES (Sigma H3375 or equivalent)
   3. Glucose (Sigma G7528 or equivalent)
   4. Sucrose (EMD Millipore 573113, VWR 57-50-1 or equivalent)
   5. 1M MgCl2 6H2O (Sigma M1028 or equivalent)
   6. 1M KCl (Quality Biological 351-044-101 or equivalent)
   7. Nuclease Free water (Ambion AM9932)
   8. 10N NaOH for pH adjustment (VWR BDH3247-1 or equivalent)
   9. 1N NaOH for pH adjustment (Sigma Aldrich S2770 or equivalent)
3. **Equipment:** 
   1. Sterile Corning 1 liter 0.22 µm filter system (Corning 28199-812 or equivalent)
   2. Sterile Corning 1 liter bottle
   3. Weigh paper
   4. Weigh boat
   5. Disposable spatula
   6. Balance (3 decimal spaces)
   7. Stir plate
   8. Stir bar
   9. Pipettor
   10. Serological pipette
   11. 3 ml disposable pipette
   12. Vacuum and tubing
   13. Graduated cylinder, 1 liter
   14. Calibrated pH meter and probe
   15. Magnetic stir bar remover
4. **Safety:**
   1. Nitrile Gloves
   2. Eye protection
   3. Lab coat
5. **Output:**
   1. 1 liter of HEPES-Sucrose Cutting Solution, pH 7.4 containing:
      1. 110 mM NaCl
      2. 10 mM HEPES
      3. 25 mM Glucose
      4. 75 mM Sucrose
      5. 7.5 mM MgCl2 6H2O
      6. 2.5 mM KCl
6. **Reference Documents:** 
   1. EQ0006 pH Meter Calibration and Usage
      1. To be Published
   2. EQ0020 Balance Calibration Validation
      1. To be Published
   3. PF0284 Measuring Osmolarity with the Vapro 5600 Osmometer
      1. To be Published

**Warning: Personal Protective Equipment (PPE) and cleanliness for RNase-free conditions should be used at all times while operating this protocol. If you are unsure what PPE you should be using, see your immediate supervisor.**

1. **Setup:** 
   1. Thoroughly clean stir bar and graduated cylinder for the preparation of this reagent. Rinse well with MilliQ water at least 3 times. Place stir bar into sterile 1 liter container.
      1. Place on top of stir plate.
   2. Ensure that balance is calibrated, refer to reference documents for assistance
   3. Ensure that pH meter and probe are calibrated, refer to reference documents for assistance.
   4. Attach the sterile corning 1 liter 0.22 µm filter system to the vacuum pump when time to filter.
2. **Methodology/Procedures:** Record all lot numbers appropriately.
   1. Using a clean graduated cylinder, measure out approximately 500 mL of Nuclease-free water and pour it into the 1 liter corning bottle with the stir bar. Recap the Nuclease-free water when not in use.
   2. Place the 1 liter corning bottle with the stir bar onto the stir plate. Turn on the stir plate to level 3.
   3. Using a calibrated balance, place down either weigh paper or weigh boat onto the balance.
      1. Press the Tare button to zero out the weight of the weigh paper or the weigh boat.
      2. Measure out 6.43 grams of NaCl into the tared weigh boat or paper. Add the NaCl to the 1 liter container while stirring.
   4. Using a calibrated balance, place down either weigh paper or weigh boat onto the balance.
      1. Press the Tare button to zero out the weight of the weigh paper or the weigh boat.
      2. Measure out 2.383 grams of HEPES into the tared weigh boat or paper. Add the HEPES to the 1 liter container while stirring.
   5. Using a calibrated balance, place down either weigh paper or weigh boat onto the balance.
      1. Press the Tare button to zero out the weight of the weigh paper or the weigh boat.
      2. Measure out 4.505 grams of Glucose into the tared weigh boat or paper. Add the Glucose to the 1 liter container while stirring.
   6. Using a calibrated balance, place down either weigh paper or weigh boat onto the balance.
      1. Press the Tare button to zero out the weight of the weigh paper or the weigh boat.
      2. Measure out 25.673 grams of Sucrose into the tared weigh boat or paper. Add the Sucrose to the 1 liter container while stirring.
   7. Using a Pipettor and a 10 ml serological pipette, measure out 7.5 mL of 1M MgCl2 6H2O. Add the 7.5 mL of 1M MgCl2 6H2O to the 1 liter container while stirring.
   8. Using a Pipettor and a 5 mL serological pipette, measure out 2.5 mL of 1M KCl. Add the 2.5 mL of 1M KCl to the 1 liter container while stirring.
   9. Let stir until the liquids and solids are completely dissolved and the reagent is homogenous with no visible solids.
   10. Obtain a calibrated pH meter and probe, for assistance with calibration and usage please refer to EQ0006 pH Meter Calibration and Usage.
   11. Place pH probe into the 1 liter container while stirring, taking care that the probe tip and the stir bar do not collide. Using a 3 ml disposable pipette, add 10N NaOH dropwise until pH nears 7.4. As you get nearer allow the reagent to equilibrate to ensure you do not add more 10N NaOH then needed.
   12. If the pH is close to 7.4, but not quite 7.4, finish adjusting pH using 1N NaOH. Take care not to overshoot the adjustment. Remove the probe store away once adjustment has been made and pH is 7.4.
   13. Using the magnetic stir bar remover, hold the stir bar in place while pouring the contents of the 1 liter container into the clean 1 liter graduated cylinder
       1. Using Nuclease-free water, bring the reagent up to the final 1 liter volume.
       2. Pour back into the 1 liter container and allow to spin.
   14. Prepare the Corning 1 liter 0.22 µm filter system by attaching the vacuum hose to the filter top. Ensure that the filter top is firmly tightened onto the 1 liter container.
       1. Slowly pour the contents of the 1 liter container into the filter top and allow to filter to completion. Discard filter top and 1 liter container when empty.
   15. Label the filtered HEPES-Sucrose Cutting solution with the name of the reagent, prep date, and the preparers initials. Store at 4°C for up to two weeks.



**Table 1**: Reagents Required for 1 liter of HEPES-Sucrose Cutting solution

1. **Take Down:** 
   1. Dispose of soiled weigh boats, weigh paper, disposable spatulas, filter tops, Corning containers in trash.
   2. Clean balance, removing all solids from the scale.
   3. Clean pH probe after use.
   4. Wipe down any surfaces with 70% EtOH that may have been soiled during preparation.
2. **Technical Information:**
   1. Unused, expired, or excess reagent can be disposed of down municipal sewer.
   2. Store HEPES-Sucrose Cutting solution for up to two weeks at 4°C