**Preparation of internal solution and ACSF** ● **TIMING 1 d**

Great care should be taken to prepare the internal solution under strict RNase-free conditions, as this will be the first solution to come into contact with the RNA sample.

Internal solution: Component Amount Final concentration (assuming a 50-ml final volume)

K-gluconate (234.25 g/mol) 1.3000 g 111 mM

KCl (74.55 g/mol) 0.0149 g 4 mM

HEPES (1 M) 500 μl 10 mM

EGTA (380.35 g/mol) 0.0038 g 0.2 mM

Mg-ATP (507.18 g/mol) 0.1014 g 4 mM

Na-GTP (523.18 g/mol) 0.0078 g 0.3 mM

Na2-phosphocreatine (255.08 g/mol) 0.0637 g 5 mM

Biocytin (372.5 g/mol) 0.2500 g 13.423 mM

**Procedure**

1. Dissolve K-gluconate, KCl, HEPES, and EGTA in ~40 ml of RNase-free water in a 125-ml Erlenmeyer flask.

**CRITICAL STEP** Ensure that all glassware, spatulas, stir bars, counters, and anything else that may come into contact with the reagents or solution are cleaned thoroughly with RNase Zap.

2. Cover the solution with aluminum foil and autoclave it. Cool the solution to room temperature before proceeding.

**PAUSE POINT** The solution can be stored at 4 °C overnight.

3. Add Mg-ATP, Na-GTP, Na2-phosphocreatine, and biocytin. Stir the contents until biocytin is completely dissolved.

**CRITICAL STEP** Ensure that all glassware, spatulas, stir bars, counters, and anything else that may come into contact with the reagents or solution are cleaned thoroughly with RNase Zap.

4. Adjust the pH to 7.25 with RNase-free 0.5 M KOH.

**CRITICAL STEP** Be sure to use a pH meter electrode that is cleaned with RNase Zap and RNase-free water before use.

5. Measure osmolarity. The osmolarity of the solution should be ~235 - 240 mOSM.

**CRITICAL STEP** Obtain at least three measurements to ensure that the readings are stable before proceeding.

aCSF: Component Amount Final concentration (assuming a 1L final volume)

NaCl (58.44 g/mol) 7.305 g 125 mM

KCl (74.55 g/mol) 0.186 g 2.5 mM

NaH2PO4 (119.98 g/mol) 0.149 g 1.25 mM

NaHCO3 (84.01 g/mol) 2.100 g 25 mM

Glucose (180.16 g/mol) 2.000 g 11.102 mM

CaCl2 (1 M) 2 mL 2 mM

MgCl2 (1 M) 1 mL 1 mM

**Procedure**

1. Dissolve NaCl, KCl, NaH2PO4, NaHCO3 and Glucose in ~900 ml of DD H2O.

2. “Bubble” the solution with carbogen 95% O2 and 5% CO2 at least 15-20 minutes.

3. Add CaCl2 andMgCl2.

4. Adjust the volume.

The aCSF can be stored at +4 °C for no longer than 4-6 days.

**Preparation of “physiologic osmolarity internal solution”**

1. Combine 494 μl of internal solution and 6 μl of RRI in an RNase-free 1.5-ml tube. Vortex the tube well.

2. After solution” that should be ~315-320 mOsm adding the RRI, measure the osmolarity of the “physiologic osmolarity internal.

**CRITICAL STEP** Compare the osmolarity of the “physiologic osmolarity internal solution” with that of aCSF. If necessary, add sucrose to aCSF until its osmolarity is 15 – 20 mOsm lower than that of “physiologic osmolarity internal solution”.

**CRITICAL STEP** Store the internal solution on ice until you are ready to begin patching.

<https://www.physiologyweb.com/calculators/ghk_equation_calculator.html>