



### Parameter

pH, Eppendorf tubes

### Sample Type

Protein Labeling Kit

### Introduction

ICPL (Isotope Coded Protein Labeling) followed by mass spectrometry allows for the relative quantification of specific proteins within complex protein mixtures. Two pH adjustments are required in the labeling process. Since sample volumes are very small, pH measurements must be made with a micro pH electrode.

### Result Statistics

# Trials	%CV
5	+/- 0.03 pH units

### Recommended Equipment

Star Benchtop pH/ISE meter (Orion 1115000); Ross glass microelectrode (Orion 8220BNWP); 0.5mL Eppendorf tubes; 10µL syringe; rack for holding tubes

### Required Solutions

pH 7.00, 10.01 and 12.46 Buffers (Orion 910107, 910110 and 910112); Filling Solution (Orion 810007); deionized water (DI); 2N NaOH. Optional: pH Electrode Cleaning Solution A for removing protein contaminants (Orion 900021)

### Solutions Preparation

Dissolve 2g of NaOH in 25mL DI Water for a 2N NaOH solution.

### Meter Setup

Connect the pH electrode to the Star Meter. Set measurement mode to pH. In Setup mode of Star Meter, set resolution to 0.01, Buffer Set to USA if using Orion buffers and read type to continuous. If all steps were followed correctly the meter display will show a number with two decimal places in the top line and "pH" to the right of the top line.

### Electrode Setup

See the electrode manual for preparation of the electrode.

### Electrode Performance Check

Check slope at least daily according to the electrode manual. Drift may be checked by comparing a 1-minute to a 2-minute reading. Results should agree with desired criteria. See troubleshooting section of manual if slope and/or drift are not acceptable.

### Electrode Storage, Soaking, and Rinsing

See electrode manual for storage 1) between measurements, 2) overnight, and 3) for long periods of time. Between measurements, rinse the electrode with DI water and blot dry. If electrode begins reading poorly or significantly slower and re-calibration does not help, try cleaning the electrode using the pH Electrode Cleaning Solution A. The solution package includes the necessary instructions for this procedure.

### Sample Preparation

Place 40µL of the sample into each Eppendorf tube. For precise measurements, allow all the standards and the samples to reach the same temperature before analysis.

### Calibration

Perform a three point calibration using pH 7.00, 10.01 and 12.46 buffers. The electrode slope should be between 92 and 102%. Read fresh portions of buffers to verify calibration. If readings are not acceptable and/or slope is not within range, see troubleshooting section of manual.

### Analysis

Rinse electrode with DI water, blot probe dry with lint free tissue, place in Eppendorf tube containing sample and measure. The pH value will be displayed. When a stable reading is achieved, the "pH" icon will stop flashing. To adjust pH to 8.5, add 0.4µL\* of 2N NaOH to sample and gently move electrode up and down in sample to mix. It is not necessary to completely remove electrode from sample vessel when adding base but moving probe to mix is critical to obtaining reproducible results. Measure pH of the sample. To adjust pH to 11.9, add an additional 0.6µL\* of 2N NaOH, mix and measure sample pH again.

### Comments

It is important to load base into the syringe very slowly to avoid air bubbles. Insert the syringe as far into the Eppendorf tube as possible before dispensing the NaOH. This ensures that all of the solution in the syringe gets into the sample.

\*Depending on the protein being analyzed, the amounts of NaOH to adjust pH may need to be fine tuned. It is recommended to begin with the amounts listed then adjust accordingly if needed.

### Quality Control (QC)

Recommended QC procedures include: calibration and calibration verification, sample duplicates, slope, and drift.