



## Parameter

pH in Tris buffer at elevated temperature

## Introduction

Tris is the most commonly used buffer for biological media. The pH value of a Tris buffer is temperature dependent. The Orion ROSS Ultra Triode will measure both the temperature and pH of the buffer with a single probe.

## Reference

R.A. Durst and B. R. Staples, Tris/Tris HCl: A Standard Buffer for Use in the Physiologic pH Range, Clinical Chemistry, Vol.18, No. 3, 1972.

## Result Statistics

See page 2

## Recommended Equipment

Orion Star or Star Plus Benchtop Meter (Cat. No. 1115000 or 1119000, or 1112000, or 1117000); ROSS Ultra Triode (Orion 8157BNUMD); Stirrer (Orion 096019). Optional: NIST calibrated thermometer; printer (Orion 1010006); Star Navigator Software (Orion 1010007) or Star Plus Navigator Software (Orion 1010017).

## Required Solutions

pH 4.01, 7.00 and 10.01 Buffers (Orion 910104, 910107 and 910110); Filling Solution (Orion 810007); deionized water (DI); ROSS storage solution (810001).

## Solutions Preparation

None Required

## Meter Setup

Connect both plugs of the triode to the Star Meter. Set measurement mode to pH. In Setup mode of Star Meter, set resolution to 0.001, Buffer Set to USA and read type to Auto. If all steps were followed correctly the meter display will show a number with three decimal places in the top line and "pH" to the right of the top line. The temperature will also be displayed in the top left of the screen. This should read the actual sample temperature rather than reference temperature (25.0°C).

## 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 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

To ensure a quick response and free-flowing junction, the sensing element and reference junction must not dry out. See electrode manual for 1) short-term storage (up to one week), and 2) long-term storage (more than one week). Between measurements, store electrode in pH 4.01 or 7.00 buffer. Rinse the electrode with DI water and blot dry with lint free tissue before measuring the next sample

## Sample Preservation

Refrigerate sample if not using immediately.

## Sample Preparation

Prepare your Tris Buffer from Tris and Tris HCl salts to the pH of choice.

## Calibration

Calibrate the probe's thermistor against a NIST calibrated thermometer if desired. Perform a three point calibration using pH 4.01, 7.00 and 10.01 buffers. The meter automatically recognizes the buffers and displays the temperature-corrected pH value for each calibration point. The electrode slope should be between 92 and 102% of the Nernst value (59.16 mV/pH unit at 25°C). Read a fresh portion of pH 7.00 buffer to verify calibration. Reading will be temperature-adjusted, see table on page 2 for expected values. If readings are not acceptable and/or slope is not within range, see troubleshooting section of manual.

## Analysis

Rinse electrode with DI water and blot dry. Collect about 50 mL of sample in a beaker. Place probe and stirrer in sample. Press the MEASURE key on the meter. The stirrer should turn on. The "pH" icon will flash until the reading is stable. Once the reading is stable, the icon will stop flashing, the stirrer will stop and the pH result and temperature will be displayed and printed.

## Quality Control (QC)

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



### pH of Tris buffer at room and elevated Temperature

	25°C	36.5°C	24.9°C
Measured pH	8.240	7.943	8.243
Expected pH*	8.240	7.941	8.243
Delta from expected pH	0.000	-0.002	0.000

\*Expected pH is calculated with a temperature coefficient of -0.026 pH unit per degree Celsius (see reference on page 1).

