



## Parameter

pH of Albumin in Tris Buffer

## Introduction

The pH of Albumin in Tris buffer is determined with the Orion 9102DJWP double junction refillable combination pH electrode by a direct read on the Orion Star Meter. This electrode is designed to avoid the possibility of introducing silver ions to the sample and minimize blockage of the reference junction when measuring pH in biological media.

## References

USP Method 791. USP29–NF24, Pg 2730. United States Pharmacopeial Convention Inc, Rockville, MD.

## Recommended Equipment

Orion Star or Star Plus Benchtop Meter (Cat. No. 1115000 or 1119000, or 1112000, or 1117000); Double junction refillable combination pH electrode with glass body and waterproof BNC connector (Orion 9102DJWP); ATC probe (Orion 927007MD); Stirrer (Orion 096019); Optional: 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 910008); deionized water (DI). Optional: pH Electrode Cleaning Solution A for removing protein contaminants (Orion 900021).

## Solutions Preparation

None Required

## Meter Setup

Connect the pH electrode, ATC probe, and stirrer to the meter. Set measurement mode to pH. In Setup mode, set resolution to 0.01, Buffer Set to USA and read type to Auto. 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. 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.

## 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 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, rinse the electrode with DI water and blot dry before measuring the next sample. 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 Preservation

Not required.

## Sample Preparation

Prepare 1L of 50mM Tris buffer, brought to pH 7.5 using HCl. Dissolve 10g Bovine Serum Albumin (BSA) in the Tris buffer to make a 1% BSA sample. Pour 50mL of sample into 100mL beaker. For best results, pour two beakers of each sample and buffer. Label one beaker as "Rinse" and use solution in that beaker to rinse electrode before measuring the sample or buffer itself.

## Calibration

Perform a three point calibration using pH 4.01, 7.00 and 10.01 buffers. 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. When using an ATC probe, compare buffer reading from calibration verification to table of buffer pH values versus temperature (see page 2). According to USP Method 791, the observed pH values must agree within 0.02 units of the expected values. If readings are not acceptable after a few iterations, consult the troubleshooting section of the electrode manual.

## Analysis

Rinse electrode, ATC probe, and stirrer with DI water. Place in a beaker containing sample for final rinse (do not blot) then move to 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.



Albumin in Tris buffer*	pH	Temperature (°C)
Sample 1	7.30	19.7
Sample 2	7.31	19.7
Sample 3	7.31	19.8
Mean	7.31	
Standard Deviation	0.006	

\*Addition of albumin decreases buffer pH from 7.5

### Temperature Corrected Values for pH 7.00 buffer

°C	0	10	20	30	40	50	60	70	80	90
pH	7.11	7.06	7.01	6.98	6.97	6.97	6.97	6.99	7.03	7.08

For a more detailed table (including buffers 4.01 and 10.01), see [http://www.thermo.com/com/cda/resources/resources\\_detail/1,2166,13217,00.html](http://www.thermo.com/com/cda/resources/resources_detail/1,2166,13217,00.html)