



Parameter

pH, 96 well plate, Star Meter

Sample Type

Albumin in Tris Buffer

Introduction

The pH of Albumin in Tris buffer in a 96 well plate is determined by a direct read with the Orion 8220BNWP Ross™ Micro pH Electrode on the Orion Star Meter.

References

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

Result Statistics

# Trials	Average
39	7.45
%CV	Analysis Time
+/- 0.02 pH units	1 minute/sample

Recommended Equipment

Star Benchtop pH/ISE meter (Orion 1115000); Ross glass microelectrode (Orion 8220BNWP); 96 well plate

Required Solutions

pH 4.01, 7.00 and 10.01 Buffers (Orion 910104, 910107 and 910110); Filling Solution (Orion 810007); 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 to the Star Meter. Set measurement mode to pH. In Setup mode of Star Meter, set resolution to 0.01 and Buffer Set to USA if using Orion buffers. 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 assembly and 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 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 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

Refrigerate sample if not using immediately.

Sample Preparation

Prepare 1L of 50mM Tris buffer, brought to pH 7.5 using HCl. Dissolve enough Bovine Serum Albumin (BSA) in 25mL of the Tris buffer to make a 1% BSA sample. Place 100µL of sample into each well of a 96 well plate. For best results fill duplicate wells for each sample to be tested so that after rinsing with DI water, the electrode can be rinsed in one well before measuring in the other. Fill duplicate control wells with 100µL of each pH buffer used in the calibration as well.

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). Re-read a fresh portion 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, place in a well containing sample for final rinse (do not blot) then move to sample and measure. The pH value and temperature will be displayed. When a stable reading is achieved, the "pH" icon will stop flashing.

Comments

If using Orion Swing Arm Holder and Stand (090043), secure the microelectrode into one of the front sections.

Quality Control (QC)

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



Results

Sample Name	pH Value
bulk	7.44
pH 4.01	4.00
pH 7.00	7.03
pH 10.01	10.00
sample 1	7.45
sample 2	7.45
sample 3	7.43
sample 4	7.44
sample 5	7.42
sample 6	7.45
sample 7	7.44
sample 8	7.45
sample 9	7.45
sample 10	7.46
sample 11	7.47
sample 12	7.47
sample 13	7.43
sample 14	7.44
sample 15	7.46
sample 16	7.46
sample 17	7.44
sample 18	7.44
sample 19	7.44
pH 10.01 (2)	9.99
pH 7.00 (2)	7.04
pH 4.01 (2)	4.03
sample 20	7.44
sample 21	7.44
sample 22	7.46
sample 23	7.46
sample 24	7.45
sample 25	7.46
sample 26	7.45
sample 27	7.44
sample 28	7.44
sample 29	7.43
sample 30	7.45
sample 31	7.46
sample 32	7.45
sample 33	7.44
sample 34	7.44
sample 35	7.43
sample 36	7.44
sample 37	7.43
sample 38	7.43
sample 39	7.43
pH 7.00 (3)	7.02
pH 4.01 (2)	4.03
pH 10.01 (3)	9.97