



### Parameter

pH, 96 well plate, Star Navigator

### 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 using the Star Navigator Software for data collection.

### 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); Star Navigator Software (Orion 1010007); 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 electrode to the Star Meter, turn the meter on and connect it to a computer. In Setup mode of Star Navigator, set resolution to 0.01 and Buffer Set to USA if using Orion buffers. Change in meter display will not occur until first measurement is made.

### 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 is 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 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 so that after rinsing with DI water, the electrode can be rinsed in one well before measuring in the other. Fill duplicate control wells containing 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 the 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 will be displayed on computer screen when electrode is stable.

### 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.



Date-Time	Sample Name	pH Value
2006-9-29 2:54 PM	bulk	7.44
2006-9-29 3:08 PM	pH 4.01	4.00
2006-9-29 3:09 PM	pH 7.00	7.03
2006-9-29 3:10 PM	pH 10.01	10.00
2006-9-29 3:11 PM	sample 1	7.45
2006-9-29 3:11 PM	sample 2	7.45
2006-9-29 3:12 PM	sample 3	7.43
2006-9-29 3:13 PM	sample 4	7.44
2006-9-29 3:14 PM	sample 5	7.42
2006-9-29 3:15 PM	sample 6	7.45
2006-9-29 3:16 PM	sample 7	7.44
2006-9-29 3:17 PM	sample 8	7.45
2006-9-29 3:18 PM	sample 9	7.45
2006-9-29 3:22 PM	sample 10	7.46
2006-9-29 3:24 PM	sample 11	7.47
2006-9-29 3:25 PM	sample 12	7.47
2006-9-29 3:26 PM	sample 13	7.43
2006-9-29 3:27 PM	sample 14	7.44
2006-9-29 3:28 PM	sample 15	7.46
2006-9-29 3:29 PM	sample 16	7.46
2006-9-29 3:30 PM	sample 17	7.44
2006-9-29 3:31 PM	sample 18	7.44
2006-9-29 3:32 PM	sample 19	7.44
2006-9-29 3:33 PM	pH 10.01 (2)	9.99
2006-9-29 3:34 PM	pH 7.00 (2)	7.04
2006-9-29 3:35 PM	pH 4.01 (2)	4.03
2006-9-29 3:37 PM	sample 20	7.44
2006-9-29 3:38 PM	sample 21	7.44
2006-9-29 3:38 PM	sample 22	7.46
2006-9-29 3:39 PM	sample 23	7.46
2006-9-29 3:40 PM	sample 24	7.45
2006-9-29 3:41 PM	sample 25	7.46
2006-9-29 3:42 PM	sample 26	7.45
2006-9-29 3:43 PM	sample 27	7.44
2006-9-29 3:44 PM	sample 28	7.44
2006-9-29 3:45 PM	sample 29	7.43
2006-9-29 3:46 PM	sample 30	7.45
2006-9-29 3:47 PM	sample 31	7.46
2006-9-29 3:49 PM	sample 32	7.45
2006-9-29 3:50 PM	sample 33	7.44
2006-9-29 3:50 PM	sample 34	7.44
2006-9-29 3:52 PM	sample 35	7.43
2006-9-29 3:52 PM	sample 36	7.44
2006-9-29 3:53 PM	sample 37	7.43
2006-9-29 3:54 PM	sample 38	7.43
2006-9-29 3:55 PM	sample 39	7.43
2006-9-29 3:56 PM	pH 7.00 (3)	7.02
2006-9-29 3:57 PM	pH 4.01 (2)	4.03
2006-9-29 3:58 PM	pH 10.01 (3)	9.97