Thermo ELECTRON CORPORATION

Print Date: 10/25/2006

Applications Log # 10A

Parameter
pH in 384 well plate

Sample Type
Aqueous samples

Introduction
The pH of an aqueous sample in a 384 well plate is determined by a direct read with the Orion 8220BNWP Ross™ Micro pH Electrode on an Orion PerpHecT® Benchtop pH Meter.
Since the electrode can be used to measure both pH and temperature with an Orion PerpHecT® Meter using the LogR feature, temperature can be recorded as well (See App Note #10B).

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

Result Statistics

# Trials	Average
10	9.92 (at 35°C)
%CV	**Analysis Time**
+/-0.02 pH units	1 minute/sample

Recommended Equipment
PerpHecT® Benchtop pH Meter (Orion 035000), Ross™ Micro pH Electrode (Orion 8220BNWP), 384-well plates

Required Solutions
pH 7.00 and 10.01 Buffers (Orion 910107 and 910110); Filling Solution (Orion 810007); deionized water (DI).

Solutions Preparation
None required

Meter Setup
Connect the electrode to the meter. Change Mode until °C, pH and mV are displayed.

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 within desired criteria. See troubleshooting 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 of different samples, rinse the electrode with DI water and blot dry before measuring the next sample.

Sample Preservation
As required

Sample Preparation
Place 50uL of sample into wells of a 384 well plate. For best results fill duplicate wells for each sample so the electrode can be rinsed in one well before measuring the other. This will ensure the most accurate readings. Fill duplicate control wells containing 50uL of each pH buffer used in the calibration as well.

Calibration
Perform a two point calibration at room temperature using pH 7.00 and 10.01 buffers. The electrode slope will be displayed and 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 dry, rinse in the first well (don't blot), and place in the second well containing sample. The pH value and temperature will be displayed, and a ready light will appear when a stable reading is achieved.

Comments
The testing done for the development of this application used pH 10.01 buffer as the aqueous sample. The pH value for this buffer at 35ºC is 9.92. Read each well plate only once. For duplicate analysis, fill and measure a separate well.

Quality Control (QC)
Recommended QC procedures include: calibration, calibration verification, slope, drift, and duplicates.

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Results

	Temperature	pH	Expected pH	Difference
Environmental Chamber	37.4	9.87	9.91	-0.04
	36.8	9.91	9.91	0.00
	35.9	9.87	9.92	-0.05
	34.3	9.87	9.93	-0.06
	34.6	9.91	9.93	-0.02
	34.6	9.91	9.93	-0.02
	35.0	9.90	9.93	-0.03
	35.0	9.90	9.92	-0.02
	34.8	9.90	9.92	-0.02
	35.0	9.90	9.93	-0.03