

Technical Note

Considerations for small volume pH measurements

Using the Orion PerpHecT ROSS Combination pH Micro Electrode

When your pH sample comes in a small container, a micro pH electrode is needed to make a convenient and accurate pH measurement. Tubes, vials, and well plates are common in the life science and biotech laboratory for holding small volumes of sample. The sample may be precious and irreplaceable, represent hours of work, be costly, or there just may be very little sample available. The ability to take a pH reading on these samples is a useful tool in the laboratory.

The Thermo Scientific™ Orion™ PerpHecT™ ROSS™ combination pH micro electrode (Cat. #: 8220BNWP) is an excellent choice for these applications. The electrode is suited for TRIS buffers and samples containing proteins, enzymes, or sulfides. The unique junction is robust and responds quickly. The diameter of the pH sensor is just 3 mm, and the necessary depth of immersion is only 4.5 mm. The table on this page displays the minimum sample volumes necessary for use in various sample containers. The stated volume ensures that the entire pH glass bulb and the reference junction of the pH electrode are submerged in the sample. This is needed to obtain a meaningful pH reading.

To measure both pH and temperature of a sample, use the Orion PerpHecT ROSS combination pH micro electrode with a Thermo Scientific™ Orion™ Versa Star Pro™ pH/LogR™ benchtop meter (Cat. #: VSTAR83). By selecting the LogR feature, temperature is determined from the resistance of the pH sensor, without the need for a separate ATC probe. The temperature is determined at the pH sensor surface itself for a truly representative reading of the sample temperature.

The Orion PerpHecT ROSS combination pH micro electrode is an excellent choice for pH samples in small containers.

If a separate ATC probe is desired, the Thermo Scientific™ Orion™ Stainless Steel Micro Automatic Temperature Compensation (ATC) Probe (Cat. #: 928007MD) can be used. Its 1 mm diameter allows for measurement in the smallest volumes. The Thermo Scientific™ Orion Star™ A211 bench meter ROSS™ micro kit (Cat. #: STARA211MCR) is recommended.

Sample containers and minimum required sample volumes

Sample container	Container size	Minimum sample volume (uL)
PCR tubes	0.2 mL	15
Micro centrifuge tubes	0.6 mL	15
Micro centrifuge tubes	1.5 mL	25
Centrifuge tubes	15 mL	50
Well plates	96 wells	20
Well plates	384 wells	15
Cryogenic storage tubes	1 mL	30
Cryogenic storage tubes	2 mL	30
Culture tubes (test tubes)	10 X 75 mm (4 mL)	40
Culture tubes (test tubes)	16 X 100 mm (14 mL)	200

5 tips for small volume measurements

Measuring small samples is a precise matter. Follow these tips to obtain an accurate pH measurement.

1. Displacement

When using a very small container (such as 384 well plates and microcentrifuge tubes smaller than 1 mL), you may notice that the level of the sample rises in the container when the pH electrode is inserted. To avoid overflow, take the pH measurement in a partially filled container.

2. Temperature response

When placing a pH electrode into a sample, the immersed portion of the sensor and the sample will equilibrate to a common temperature. If the pH electrode and sample are at different temperatures, more time will be required. Allow time for the temperature to stabilize before taking the pH measurement.

3. Dilution effect

When taking the pH of a sample in a small container, a few small drops of purified rinse water on the pH probe may have a perceptible effect on the volume or pH of the sample. Remove excess drops of water by one brief, gentle touch of a wiper. Don't rub or use pressure.

4. Thermal mass

When testing the pH in a small container, the thermal mass of the pH probe will be large compared to the thermal mass of the sample. For example, if the sample is cold, the sample temperature will rise when the warmer room temperature pH probe is inserted. If this temperature effect is not desired, take measures to equilibrate the pH probe to the sample temperature prior to testing.

For example, place a beaker of purified water and a rinse bottle of purified water into the temperature zone of the sample. That might be a refrigerator, water bath, or ice bath, etc. Allow time for them to come to temperature. When ready for testing, place the pH probe into the beaker of purified water and wait for it to come to temperature. Then remove excess water by one brief, gentle touch of a wiper, and place the probe directly into the sample. Take the pH and temperature readings when stable. After the measurement, rinse the probe with the rinse bottle and place back into the beaker. Repeat the process using the temperature-adjusted beaker and rinse bottle until the testing is done.

5. Container shape

Containers with a conical or U-shaped bottom will need less volume for a good pH measurement than a flat bottom container.

Ordering information

Product	Description	Catalog number
Electrode and Probe	Orion PerpHecT ROSS Combination pH Micro Electrode	8220BNWP
	Orion Stainless Steel Micro Automatic Temperature Compensation (ATC) Probe	928007MD
Meter and Meter Kit	Orion Versa Star Pro pH/LogR Benchtop Meter	VSTAR83
	Orion Star A211 Bench Meter ROSS Micro Kit	STARA211MCR

Learn more at thermofisher.com/8220BNWP

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