INSTRUCTIONS FOR FINESSE TRUFLUOR pH SENSOR USER ENTERED 2 POINT CALIBRATION

January 3, 2012

Range of 2 Point TruFluor pH Calibration

In order to ensure optimal performance from the Finesse TruFluor pH sensors it is recommended that a user entered 2 point calibration is performed upon initial use followed by daily one point standardizations.

This procedure assumes that the target operating pH setpoint is below the pH of the media as received. Most media are intentionally chosen to be more basic by 0.25 to 0.45 pH units in order to allow for CO_2 sparging in the incipient growth phase. It is important to follow the "TruFluor pH QuickStart Guide" and make sure that the pH sensor has been submerged in media for at least 4 hours (and preferably 6 hours) before performing the 2 point calibration and that the bioreactor and its contents are at the operating temperature of the initial phase.

The recommended method for performing a 2 point calibration in a typical bioreactor configuration is to take an initial off-line sample with the media at its upper pH value (0.25 to 0.4 pH units above the set point) and to use this as the first calibration point. The next step is to sparge CO_2 into the system until the pH is sligthly below the target pH set-point. Optimally this lower pH value will be 0.25 to 0.4 pH units below the target operating pH value. The system should be allowed to come to steady state for at least 5 minutes after changing the pH set point; then a second off-line sample can be taken and used as the second calibration point for the TruFluor pH sensor.

Performing an initial 2 point calibration should ensure that your TruFluor pH sensors meet specification. The 2 calibration points should separated by 0.3 to 0.8 pH units and centered on the final target pH control set-point. . A pH separation of ~ 0.6 pH units between the upper and lower pH calibration points is close to ideal. Running calibration points having a span of less than 0.3 pH units around the final target operating pH will still ensure correct operation of the TruFluor pH sensor within the range of the two point calibration but the sensor may not meet specification when running outside of the range where the two calibration points were taken and is therefore not best practice. Taking calibration points further away than 0.4 pH units from the target operating pH value (i.e., a span exceeding 0.8 pH units) has the potential to skew the calibration scaling so that it will not read correctly at the set-point until the first 1 point offset is performed and is therefore also not considered best practice.

Finesse Solutions, Inc. – Proprietary & Confidential – This document is the property of Finesse Solutions, Inc. and contains proprietary and confidential information. Do not duplicate it without prior written authorization by Finesse Solutions, Inc.

Example process setup time-line for pH two-point calibration only

1. **Time Zero:**
    a. Media loaded into bioprocess container (BPC), with all DO and pH sensors connected.
2. **Zero to 2 hrs:**
    a. Agitation started, system temperature control started
3. **5 to 6 hrs:**
    a. System at temperature (e.g., 37.5°C)
    b. Media sample taken for first point of a 2 point TruFluor pH calibration based on offline analysis. First point value is applied to TruFluor pH calibration. First point is ideally 0.25 to 0.4 pH units above the operating pH set point.
    c. Sparge CO_2 so that the pH value is 0.25 to 0.4 pH units below target operating pH set point.
4. **6.5 to 7 hrs:**
    a. Stop all sparging, let pH stabilize and take second offline sample and apply of 2nd point of TruFluor pH calibration as well as 1 point offset if using traditional pH electrode.
    b. Enable system pH and DO control to reach target set-points, and seed reactor when all set-points are achieved and stable.
5. **30-31hrs +:**
    a. Take solution sample for offline analysis and apply 1st daily one point offset for TruFluor pH.

Example process setup time-line for pH and DO two-point calibration

Please note that a 2 point calibration on the DO is not necessary under typical operating conditions. If the system has been fully sparged with Nitrogen and the reading of the TruFluor DO is ≤0.5 % Sat, there is no reason to perform a 2 point calibration on the TruFluor DO.

1. **Time Zero:**
    a. Media loaded into bioprocess container (BPC), with all DO and pH sensors connected.
2. **Zero to 2 hrs:**
    a. Agitation started, system temperature control started
3. **4 hrs:**
    a. N_2 sparged to system to remove DO for 2 point DO calibration
4. **5 to 6 hrs:**
    a. System at temperature (e.g., 37°C)
    b. 1st DO calibration point taken (DO zero point)
    c. N_2 shut off, Air sparged to system for second calibration point
5. **6 to 6.5 hrs:**

Finesse Solutions, Inc. – Proprietary & Confidential – This document is the property of Finesse Solutions, Inc. and contains proprietary and confidential information. Do not duplicate it without prior written authorization by Finesse Solutions, Inc.

a. Second DO (100% air saturated) calibration point taken
b. Media sample taken for first point of a 2 point TruFluor pH calibration based on offline analysis. First point value is applied to TruFluor pH calibration. First point is ideally 0.25 to 0.4 pH units above the operating pH set point.
c. Sparge CO_2 so that the pH value is 0.25 to 0.4 pH units below target operating pH set point.

6. 6 to 7 hrs:

a. Stop all sparging, let pH stabilize and take second offline sample and apply of 2nd point of TruFluor pH calibration as well as 1 point offset if using traditional pH electrode.
b. Enable system pH and DO control to reach target set-points, and seed reactor when all set-points are achieved and stable.

7. 30-31hrs +:

a. Take solution sample for offline analysis and apply 1st daily one point offset for TruFluor pH.

Finesse Solutions, Inc. – Proprietary & Confidential – This document is the property of Finesse Solutions, Inc. and contains proprietary and confidential information. Do not duplicate it without prior written authorization by Finesse Solutions, Inc.