Calibration Check

Please read all instructions prior to starting the procedure.

Required Materials Check List

- CF-8 Calibration Kit
- PR-1 Reconditioning Kit
- Manual 0.5 to 10 uL 8-channel pipettor
- 0.1-10 uL low retention pipet tips (Fisher cat # 02-717-134)
- 200 uL Single Channel Pipettor and tips
- 8-well PCR strips (2)
- Low lint lab wipes
- dH_20

Refer to the Tip at the bottom of the page regarding the importance of using shorter, rigid tips.

Cleaning Instructions

1. Open the vial containing PR-1 and use the applicator provided in the kit to remove a pin-head sized amount of the compound.
2. Apply a very thin, even layer of PR-1 to the surface of the upper and lower pedestals.
3. Wait 30 seconds for the PR-1 to dry.
4. Fold a clean, dry laboratory wipe into quarters and remove the PR-1 by aggressively rubbing the surface of the upper and lower measurement surfaces until all the black compound residue is removed and an aliquot of water "beads up" on the surface as shown in *Figure 1*.

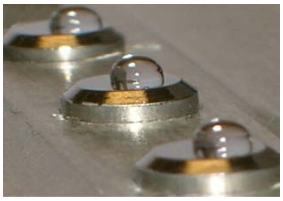

Figure 1

Sample Loading Hints

- Position the instrument at an angle that will allow for optimal use of the pipette guide (Figure 2).
- Ensure the NanoDrop 8000 is not situated near an air vent or an exhaust fan from a nearby instrument.
- Use a small-volume (0.5-10 uL) manual 8-channel pipettor to load both the dH_20 and the CF-1 aliquots.

Tip: The use of electronic pipettors is not recommended for this procedure.

- Use shorter, more rigid pipette tips such as the 0.1 -10 uL low retention tips from Fisher, catalogue # 02-717-134 for optimal results. The tips MUST fit tightly on all 8 positions of the pipettor.
- Always change pipette tips after each set of measurements.
- Ensure that adequate volumes of sample are being pipetted onto the center of each pedestal.

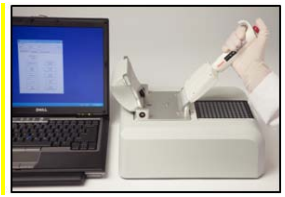

Figure 2

Discharge and Touch Sample Delivery Method Practice

It is **very important** to deliver the CF-1 aliquots to all pedestals in a single motion using a multi-channel pipettor. It is highly recommended that the steps below be practiced using water before starting the calibration check procedure.

1. When the pipette tips are close to the measurement pedestals, discharge the fluid and allow the drops to hang on the end of the tips.
2. Gently touch the droplets to the pedestals and allow them to be pulled off the tips and onto the pedestals by surface tension (Figure 3).

Figure 3

3. For additional information,

Tip: *It is important to use tips with a rigid structure to ensure the tips do not splay or skew when delivering the samples.*

Tip: *It is suggested that this technique be practiced until consistent delivery to all 8 positions is routine and quick.*

For additional information, please check the *NanoDrop 8000 Calibration Check Best Practices* tutorial, located on our website, at http://www.nanodrop.com/NanoDrop8000_Calibration_Check_Best_Practices/index.htm .

Calibration Check Procedure

1. Use PR-1 to clean and recondition the pedestals as described on page 1.This step must be completed prior to beginning the calibration check procedure.
2. Select the Calibration Check Module: Tools & Configuration Tab > Utilities & Diagnostics > Calibration Check
3. Add 55 uL of dH_20 to each well of one of the 8-well strips provided. Do **NOT** aliquot the CF-1 at this time.
4. Use an 8-channel pipettor to simultaneously pipette 1.5 uL of water to each pedestal position, lower the arm and click **OK** to initialize the instrument**.**
5. Use a laboratory wipe to remove the water aliquots from **both** the upper and lower pedestals and **change tips**.

   Tip: *It is important to use tips with a rigid structure to ensure the tips do not splay or skew when delivering the samples.*
6. Use a manual 8-channel pipettor to simultaneously pipette fresh 1.5 uL aliquots of water to each pedestal position, lower the arm and click **Blank.**
7. After the measurement is complete, use a lab wipe to remove the water aliquots from all 8 upper and lower pedestals and **change pipette tips**.
8. Enter the Target Absorbance of the CF-1 in the pop-up box “Target absorbance @350 nm 1 mm pathlength.”

   Tip: *The lot specific target absorbance is located on the CF-1 ampoule label. Ensure the value for use with the NanoDrop 8000 is entered.*
9. Thoroughly mix the CF-1 Calibration Fluid by vigorously shaking the ampoule and then hold the ampoule upright and tap lightly to ensure all of the CF-1 solution is collected in the bottom portion of the ampoule.
10. Carefully break the neck of both ampoules of CF-1 Calibration Fluid included in the CF-8 kit and equally dispense the contents of both ampoules into the wells of the second 8-well PCR strip.
11. Use a manual 8-channel pipettor to simultaneously draw up eight 1.5 uL aliquots of CF-1

    Tip: *When drawing CF-1 solution up into pipette tips (1.5* uL*), quickly check by visual inspection that each tip contains equal amounts in all 8 pipette tips. If one or more tips is missing CF-1 or has less volume than the other tips, discard the CF-1 and tips. Using a new set of tips, draw up 8 fresh aliquots of CF-1 (1.5 uL each).*
12. Use the **Discharge and Touch** method as described on page 1 to simultaneously pipette 1.5 uL of CF-1 onto each of the eight bottom pedestals, lower the arm, and click the Measure button.

    Tip*: Very quickly visually check that each pedestal has an equal amount of CF-1 and the droplets are centered on the pedestals. If one or more pedestals has a smaller volume of CF-1 or the CF-1 is not centered on each measurement pedestal, wipe the CF-1 away, discard the pipette tips and draw up 8 fresh aliquots of CF-1 (1.5 uL each).*

    Tip*: If the visual inspection takes more than a few seconds, wipe the CF-1 away, discard the pipette tips, use a new set of tips and draw up 8 fresh aliquots of CF-1 (1.5 uL each). Allowing the CF-1 to remain on the pedestals even for a short period of time (eg: 15-20 seconds) will allow the CF-1 to start concentrating and may result in higher than expected absorbance values.*
13. After the measurement is complete, use a lab wipe to remove the CF-1 from the **both** upper and lower pedestals, **change tips** and repeat steps 11 & 12 for a total of 5 sets of measurements.