Calibration range (10-fold serial dilutions)

6. Dispend 180 µL of diluent in a new tube (tube number 2).

8. Homogenize the initial re-hydrated DNA tube by gently mixing for 5 seconds. Briefly centrifuge it.

9. Pipet 20 µL of re-hydrated DNA and transfer it to tube number 2.

10. Homogenize tube number 2 by mixing for 5 seconds then centrifuge it briefly.

11. Repeat these steps to prepare tubes 3, 4, etc.

These DNA solutions are stable for 72 hours stored at 5°C ± 3°C.

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Primary Measurement Standard

Of *Legionella pneumophila* DNA

Notice

Reference: SRM_LEGDNA_01

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Storage

Primary Standard DNA and diluent must be stored at **-20°C ± 3°C** .

Equipment and consumables required but not provided

- Sterile and « nuclease free » 1.5 mL microtubes
- Calibrated micropipettes
- Sterile and « nuclease free » filter tips
- Mini bench centrifuge
- Bench mixer

Important

- ✓ The dehydrated DNA is to be re-hydrated **with the provided diluent.**
- ✓ Primary Standard DNA of *Legionella pneumophila* must be used by personnel observing **Good Laboratory Practice** and using **calibrated laboratory equipment**.
- ✓ Change the filter tip between each pipetting to avoid calibration deviations.
- ✓ We recommend not to pre-wet the tip before pipetting.

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Operating instructions

1. Defrost the diluent tube at room temperature. Homogenize the diluent by mixing for 5 seconds. Then centrifuge it briefly.

2. Take the dehydrated DNA tube and centrifuge it for 10 seconds.

3. According to desired concentration and the loading volume of the PCR system you use, calculate the diluent volume to add in order to re-hydrate the DNA:

V diluent = [(1.06e7 (GU/tube) / desired C_{DNA} (GU/well)] X V loading (µL/well)

- ➢ To obtain a DNA solution of **250 000 GU / well with 5 µL of sample**, add :

Diluent volume = [(1.06e7 (GU/tube) / $2.5^{e}5$ (UG/well)] X 5 (µL/well) = **212.5 µL**

- ➢ To obtain a DNA solution of **250 000 GU / well with 6 µL of sample**, add :

Diluent volume = [(1.06e7 (GU/tube) / $2.5^{e}5$ (GU/well)] X 6 (µL/well) = **255 µL**

4. Add the calculated diluent volume to the dehydrated DNA tube.

5. Let stand for **1 hour ± 5 minutes** at **5°C ± 3°C** without homogenizing.

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