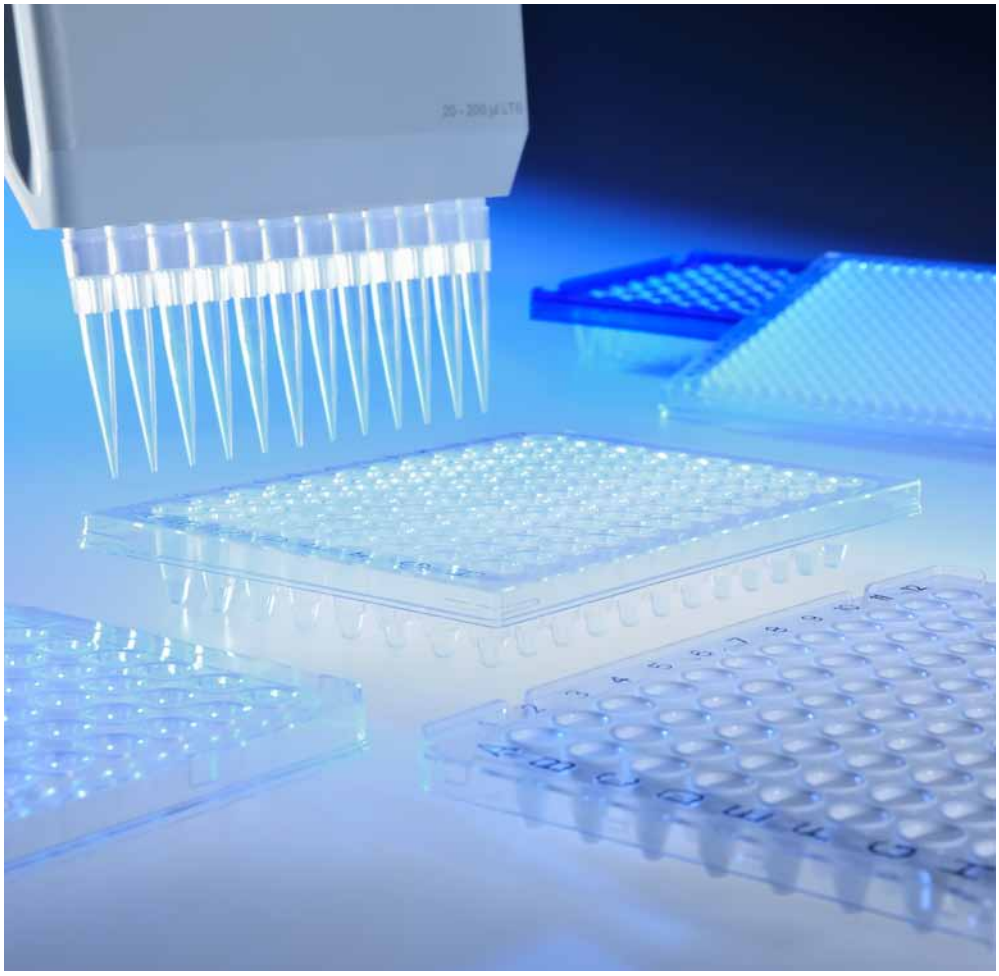
Application Note

Trust your qPCR Results

With Good Pipetting Techniques



This application note describes how Good Pipetting Techniques enhance the accuracy and reproducibility of qPCR results by minimizing pipetting errors when generating a standard curve.

METTLER TOLEDO

Pipetting Errors Invalidate qPCR Results

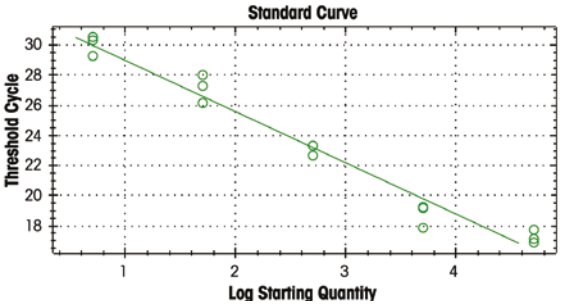
In our study we show that Good Pipetting Techniques are vital to generate accurate and valid standard curves, which are a critical component of a qPCR assay.

Real-time-polymerase-chain reaction technique

Real-time-polymerase-chain reaction or quantitative real time PCR is a powerful technique for the quantitative analysis of nucleic acid samples, involving the simultaneous amplification and quantitation of a targeted DNA molecule.
A critical component of the assay is generating a standard curve based on samples of known concentration, typically through serial dilutions of a stock. Because each serial dilution involves a pipetting step, pipetting errors will accumulate across the dilution series, completely invalidating the experiment by altering the reported amplification efficiency*.

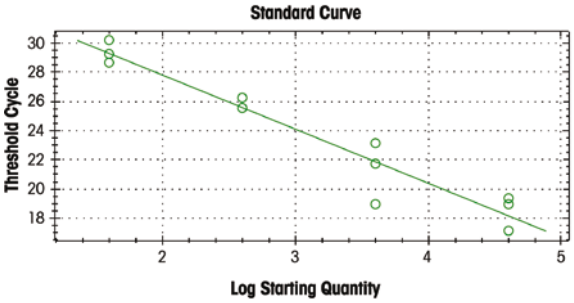
Effects of pipetting errors on qPCR results

Rainin investigated the effect of systemic pipetting errors on qPCR experiment validity using 10-fold serial dilutions of human DNA for the standard curve. A pipetting error of -20% per pipetting cycle resulted in an efficiency of 86% (Fig. 2), while a +20% error produced an efficiency of 118.7% (Fig. 3). With results like these, an experiment would need to be rerun because neither standard curve would be considered valid.
With the correct pipetting techniques, the amplification efficiencies of the generated qPCR standard curve are typically 95% to 105% (Fig. 1), and the results are therefore valid.



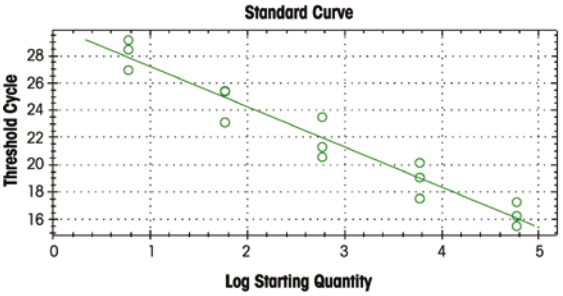
Efficency = 97.0% **Slope = -3.396**

Figure 1: Correct pipetting techniques
qPCR standard curve generated with correct pipetting at every step in the dilution series. Amplification efficiencies are typically 95-105%.



Efficency = 86.0% **Slope = -3.709**

Figure 2: -20% pipetting error
qPCR standard curve generated with a -20% pipetting error at every step in the dilution series. Amplification efficiencies are typically <90%.



Efficency = 118.7% **Slope = -2.942**

Figure 3: +20% pipetting error
qPCR standard curve generated with a +20% pipetting error at every step in the dilution series. Amplification efficiencies are typically >110%.

*To be considered valid, a qPCR experiment must reveal amplification efficiency of 90-110%. Over- or under-efficiency indicates serious experimental flaws in the assay.

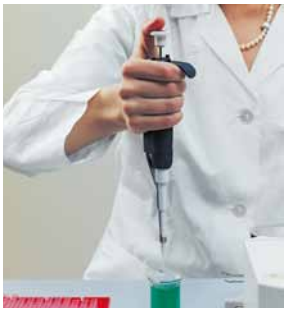
Tips and Tricks for Good Pipetting Practice

Consider four tips for Good Pipetting Practice when generating a standard curve based on samples of known concentration and get valid results.

Tip 1 - Immersion Angle

Keep the immersion angle of the tip as close to vertical as possible—no greater than a 20-degree angle from vertical. Aspirating at an angle greater than this can affect sample accuracy by up to 0.5% (and up to 2.5% in micro-volume pipettes).

Incorrect immersion



Correct immersion

Tip 2 - Immersion Depth

When immersed too deeply, air pressure can force liquid into the tip causing more sample volume than desired to be aspirated. When the tip isn't immersed deeply enough, air can be aspirated into the tip. This will prevent the pipette from accurately aspirating the selected volume and can also result in sample splash-up.

Pipette volume range	Immersion depth
0.1 – 10 µl	1 – 2 mm
10 – 200 µl	2 – 3 mm
200 – 2000 µl	3 – 6 mm
> 2000 µl	6 – 10 mm

Tip 3 - Aspiration Rate Effects

Releasing the pipette plunger too quickly can cause several technique- related problems, including splash up. After releasing the plunger button in a smooth and controlled way, it is necessary to pause briefly before withdrawal from the sample ensuring that the full volume of sample is drawn into the tip.

Tip 4 - Consistency

Achieving high accuracy and precision requires consistency in your pipetting technique. Excessively fast aspiration can lead to splashing, aerosols, contamination of shaft and piston, and even volume loss of the sample. When depressing and releasing the plunger, maintain a consistent rhythm, speed, and technique.

For more tips and tricks:

▸**www.mt.com/Pipetting-Technique**

Rainin Solutions Improve Data Accuracy

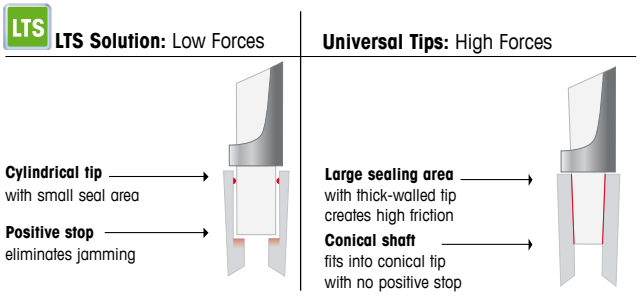
Good pipetting technique is as important for ensuring accurate results as selecting the right pipette and tips, and servicing the pipette regularly.

Regular Service

Like all things mechanical, a pipette is constructed with mechanical parts that won't last forever. For example, the surface of the piston can deteriorate over time, causing seal failure and inaccuracy or sample contamination. Rainin's program for regular calibration and preventive maintenance will reduce or even prevent out-of-tolerance failures.

LTS Pipettes for consistent sample pick-up

Whether you're using a single channel or multichannel pipette, Rainin's patented LiteTouch System (LTS) assures consistent sample pick-up every time. Unlike universal tips, which rely on force and friction to mount tips onto the pipette, LTS's cylindrical tip and positive stop assures a secure and consistent fit with minimal tip mounting force and eliminates tip mounting force as a variable.as a variable.



Rainin Instrument, LLC
a METTLER TOLEDO Company
7500 Edgewater Drive
Oakland, CA 94621
Phone: 510 564 1600
Fax: 510 564 1733

www.mt.com/rainin
For more Information