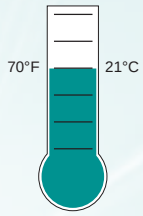
Handheld Pipetting – Best Practices

1 Before You Start

Ensure Temperature Equilibrium

The pipette, tips and liquids need to be equilibrated to room temperature if the experiment allows.

Temperature differences lead to volume contraction or expansion of the air cushion inside the pipette tip and pipette, which can negatively impact the accuracy and precision of the dispense.

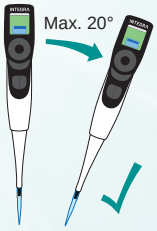


2 How To Pipette

Maintain Consistent Pipette Angle

Whenever possible, hold the pipette at a consistent angle throughout the entire pipetting process. The angle should not exceed 20 degrees.

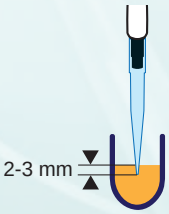
With changing the angle, the hydrostatic pressure inside the tip varies. As a result, the aspiration volume will be inconsistent.



How To Aspirate

It is best to immerse the pipette tips just below the liquid's surface (2-3 mm) to allow the desired volume to be aspirated.

Immersing the pipette tip too deeply increases the risk of liquid droplets clinging to the outside of the pipette tip. Liquid retained on the outside of the tip can result in an inaccurate dispense.



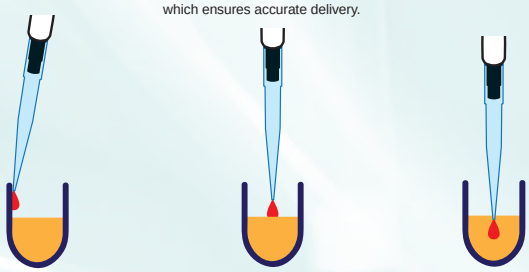
Touch Off After Dispense

After a dispense, use one of the following 3 methods to remove the pipette from the target vessel.

Side Wall Touch Off
(Standard Method)
Remove the pipette tip by sliding the tip end along the sidewall of the vessel. Recommended to achieve the most accurate dispense.

Surface Touch Off
Remove the tip by touching off the liquid droplet on the surface of the liquid in the container. Recommended when dispensing less than 1µL as a neat transfer. Touching the droplet to the liquid draws the small droplet out of the pipette tip which ensures accurate delivery.

Into Liquid Dispense
If the dispense was made directly into the liquid it is considered a wet-dispense and a touch off is not required. Ideal for small volume dispensing to ensure the liquid does not remain on the side of the vessel.



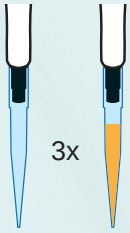
After a dispense, residual liquid often clings to the tip end. The following techniques remove this residual liquid from the tip.

3 Optimizing Pipetting Performance

Pre-Wet

After loading tips onto your pipette, aspirate and dispense the nominal volume 3 times. This will equilibrate temperature differences and humidify the dead air space inside the pipette and tip.

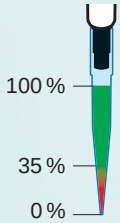
When neglecting the pre-wetting procedure, the first few dispenses tend to deliver less volume due to evaporation. The evaporation can also cause droplet formation on the tip end, as vapor pressure increases and liquid is forced outside the tip.



Optimize The Volume Range

Air displacement pipettes show the best performance between 35 % and 100 % of the nominal volume.

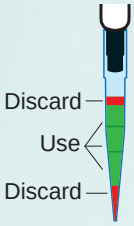
Pipetting within the optimal volume range is less technique dependent and reduces user related pipetting errors.



Discard First And Last Dispense

When dispensing multiple aliquots, it is recommended to discard the first and last dispense of the series.

These two dispenses should not be used for the assay because they contain the largest errors. It is especially important to discard a last dispense as this dispense includes the accumulated error of all previous dispenses.



Pipetting Viscous Liquids

Viscous samples should be aspirated and dispensed at slower speeds and in "Reverse pipet" mode.

The small pipette tip orifice and the elasticity of the air column prevent viscous samples from being aspirated and dispensed at fast speeds. Viscous liquids also adhere to the tip's inside wall, making it difficult to completely empty the pipette tip. Reverse pipette mode aspirates the selected volume plus an extra dispense to compensate for the retained liquid. The extra dispense is discarded.

Pipetting Volatile Liquids

Pre-wet pipette tips to humidify the dead air space. Volatile solutions should be pipetted quickly and in "Reverse pipet" mode.

This minimizes evaporation. The reverse pipette mode incorporates a larger sample volume to minimize the effect of evaporation on the actual volume to be delivered.

Calibrate Based On Liquid Density

Significant pipetting errors can occur when liquids other than aqueous solutions are used. Recalibrate your pipette if the liquid has a considerably different density than water.

Pipettes are normally tested and calibrated at the factory with distilled water at room temperature. Pipetting liquids with different densities results in inaccurate dispenses.

INTEGRA Biosciences AG
7205 Zizers, Switzerland
T +41 81 286 95 30
F +41 81 286 95 33
info@integra-biosciences.com

INTEGRA Biosciences Corp.
Hudson, NH 03051, USA
T +1 603 578 5800
F +1 603 577 5529
info-us@integra-biosciences.com

INTEGRA Biosciences Deutschland GmbH
78467 Konstanz, Germany
T +49 7531 3615 815
F +49 7531 3615 819
info-de@integra-biosciences.com

INTEGRA Biosciences SAS
95062 Cergy-Pontoise Cedex, France
T +33 (0)1 34 30 76 76
F +33 (0)1 34 30 76 79
info-fr@integra-biosciences.com

INTEGRA Biosciences Ltd.
Egham, Surrey TW20 9EY, UK
info-uk@integra-biosciences.com

INTEGRA
www.integra-biosciences.com