

Pipetting to avoid bubbles

Careful pipetting is crucial in obtaining accurate test results when performing any ELISA test. Sometimes air, resulting in bubbles, can be drawn into the pipette or dispensed into the wells. If this happens, bubbles can influence optical density values and results. To minimize or eliminate this problem, reverse pipetting is recommended for the addition of reagents to the ELISA plate.

Reverse pipetting with a multichannel pipette:

- 1. Put new tips on the pipette, ensuring they are on tight and straight.
- 2. Press the plunger past the first stop and halfway to the second stop.
- 3. Draw the liquid in a slow motion, being careful that no air bubbles are drawn into the tip. Check for consistency of volume in the tips.
- 4. Touch the tips to the edge of the reagent reservoir to remove excess liquid on the outside of the tips.
- 5. If the wells on your plate are empty, position the tips into the lower corner of the wells.
- 6. If the wells on your plate contain liquid, position the tips above the liquid.
- 7. Slowly dispense the liquid into the wells by depressing the plunger to the first stop. Be careful not to splash liquid out of the wells, and make sure there are no drops left on the tips.
- 8. To repeat, hold the plunger at the first stop and continue with step 3.
- 9. Eject the tips into an appropriate waste container.

Note: Reverse pipetting uses more reagent/volume (="dead volume").