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Requirements and Eppendorf Solutions for Immunological Analyses

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How your Pipetting System Can Protect your ELISA Analysis

You know how crucial dosing accuracy is to your ELISA assay: Any potential dosing error – e.g., due to using non-calibrated pipettes or alternative pipette tips – has the potential to impact your entire analysis. ELISA assays comprise many critical steps that go beyond liquid additions to the plate. Many ELISA kits, for instance, require dilution of certain reagents – such as standard, control and conjugate – before starting the analysis. Low OD values can occur due to inhibition from residues after washing steps were completed or due to dispensing errors. The keys to achieving dosing accuracy lie in a calibrated pipetting system and good pipetting technique (see Table 1).

Table 1: Typical problems with ELISA due to uncalibrated liquid handling tools, poor tip quality and pipetting technique

Problem	Affected by	Solution
Nonspecific color development	Cross-contaminated wells	Pipette slowly and carefully. Use a new tip for each dispensing step.
	Inaccurate dosing	Only use calibrated liquid handling tools. Use original pipette tips; have pipette calibrated with alternative pipette tips.
Low precision	Varying well volumes	Only use calibrated liquid handling tools. Use original pipette tips; have pipette calibrated with alternative pipette tips.
	Reagent splashes during additions, cross-contamination	Pipette slowly and carefully.
Low OD values	Pipetting errors	Apply good pipetting technique. Only use calibrated liquid handling tools. Use original pipette tips; have pipette calibrated with alternative pipette tips.
Nonlinear standard curve	Dosing error when diluting the standard solution	Apply good pipetting technique. Use original pipette tips; have pipette calibrated with alternative pipette tips.

Overall Requirements for Liquid Handling Tools

Immunological analyses play an important role in diagnostics and employ a broad range of methods depending on whether an analysis is qualitative, semi-quantitative or quantitative. The most frequently performed assays in diagnostics, ELISA-based tests, place three essential requirements on liquid handling systems: dispensing accuracy, contamination prevention and chemical resistance. While chemical resistance relates more to the integrity of the liquid handling tool, dispensing accuracy and contamination directly affect analyses. If the system does not meet the requirements, nonspecific color development, low OD values, reduced precision or nonlinear standard curves may occur in the analysis. Multi-channel pipettes allow fast and easy filling of 96- and 384-well plates, but laboratories still need to regularly maintain and calibrate their liquid handling tools and use high-quality tips to ensure dosing accuracy. Reliably controlling movement of liquids is also crucial for achieving accurate ELISA tests, and slow pipetting reduces the risk of contamination. Using good filter tips with two layers or different liquid handling tools for dispensing substrate and conjugate during ELISA tests are also advantageous. Surfaces without large depressions or grooves make chemical decontamination much easier. Moreover, liquid handling tools that are not made with processed metal are ideal for performing ELISA tests as many of the chemicals used in the process will corrode metals.

Eppendorf product solutions

Requirement	Key aspect	Recommended Eppendorf product		Healthcare diagnostic work
		Liquid handling system: Air-cushion	Liquid handling system: Positive-displacement	Immunological Analyses (Quantitative ELISA)
Dosing accuracy	**Maintenance and calibration:** Close-meshed inspection ensures high dosing accuracy.	> epServices for all pipettes	> epServices for all Multipette® multi-dispensers	
	Manual control of liquid movement: Smooth and balanced stroke of the operating button allows precise dispensing control.	> Eppendorf Reference® 2 family > Eppendorf Research® plus family	> Multipette® M4	
	Electronic control of liquid movement: Electronically controlled liquid aspirations and dispensings at predefined speeds allow maximum control.	> Eppendorf Xplorer® family	> Multipette® E3(x)	
	Challenging liquids: Increased accuracy for safe transfer of challenging liquids (foaming, viscous, aggressive)	> Eppendorf Xplorer® family with liquid adjustment > Eppendorf Xplorer® family with liquid types managed via Pipette Manager	> Multipette® M4 > Multipette® E3(x)"	
	LH device and tips are a system: Using original pipette or dispenser tips enhances the reproducibility of pipetting results with maximum precision and accuracy.	> epT.I.P.S.® pipette tips	> Combitips® advanced dispenser tips	
	Dispenser tips: Integrated piston wipes the liquid from the inner surface of the tips during dispensing.	*not applicable due to construction principle (air-cushion)*	> Multipette® M4 > Multipette® E3(x)	
Contamination prevention	**Aerosol accumulation in the pipette cone:** Single-button operation of pipettes reduces aerosol-carrying air flow into the pipette cone.	> Eppendorf Reference® 2 family	*not necessary: sample is hermetically sealed within Combitips® advanced due to construction principle (positive displacement)*	
	Dispenser tips: Sample is hermetically sealed within the dispenser tip without aerosol formation.	*not applicable due to construction principle (air-cushion)*	Combitips® advanced for > Multipette® M4 > Multipette® E3(x)	
	Long-distance pipette tips: Select tip shape according to the vessel (e.g. Vacutainer®) to ensure easy access to your sample when working with deep, slim vessels.	> epT.I.P.S.® 5 mL L > epT.I.P.S.® 1,250 µL L	*not available*	
	Tips wrapping: Using individually sterile-packed tips helps to avoid contaminating the rest of a tip box.	> epT.I.P.S.® Singles	> individually blister-wrapped Combitips® advanced	
	Pipette filter tips: Filters of EPA class 12 according to ISO 1822 (equivalent to ISO 25 E according to DIN EN ISO 29463-5) prevent the entry of aerosols and biomolecules into the pipette cone.	> ep Dualfilter T.I.P.S.® > ep Dualfilter T.I.P.S.® SealMax®	*not necessary: sample is hermetically sealed within Combitips® advanced due to construction principle (positive displacement)*	
	Manual control of liquid movement: Smooth and balanced stroke of the operating button allows precise dispensing control.	> Eppendorf Reference® 2 family > Eppendorf Research® plus family	> Multipette® M4	
	Electronic control of liquid movement: Electronically controlled liquid aspirations and dispensings at predefined speeds allow maximum control.	> Eppendorf Xplorer® family	> Multipette® E3(x)	
	Purity of pipette and dispenser tips: Purchasing tips in required and externally certified purity from manufacturers ensures sample safety.	> epT.I.P.S.® in: PCR clean, PCR clean and sterile > ep Dualfilter® T.I.P.S. in: PCR clean and sterile > ep Dualfilter® T.I.P.S. SealMax® in: PCR clean and sterile	Combitips® advanced in: > PCR clean > Biopur	
Decontamination	**Pipette/Dispenser autoclavable**	> Eppendorf Reference® 2 family > Eppendorf Research® plus family > All lower parts of Eppendorf Xplorer® family	*not applicable*	
	Pipette/Dispenser tips autoclavable	> epT.I.P.S.®	*not applicable*	
	Tips box/rack autoclavable	> epT.I.P.S.® Box (2.0)	> Combitips® advanced Rack (without consumables)	
	Using decontamination agents: Broad chemical resistance to common decontamination agents facilitates decontamination of devices.	> Eppendorf Reference® 2 family > Eppendorf Research® plus family > All lower parts of Eppendorf Xplorer® family	Decontamination with alcohol recommended > Multipette® M4 > Multipette® E3(x)	
	Advanced surface robustness: PTFE in surfaces strengthens cleaning and decontamination properties.	> Eppendorf Reference® 2 family > Eppendorf Research® plus family	Surface does not contain PTFE.	
	Smooth surface: Surface without interrupted surfaces or recesses enable easy and effective wipe disinfection.	> Eppendorf Reference® 2 family	*not applicable*	
Chemical resistance	**Robust chemical resistance**	> Eppendorf Reference® 2 family (1), [6] > Eppendorf Research® plus family (1), [7] > All lower parts of Eppendorf Xplorer® family	> Multipette® M4 [8]	
	Option: Advanced chemical resistance	Special lower part available with resistance to highly aggressive chemicals (e.g. TFA) > Eppendorf Reference® 2 variants (2) > Eppendorf Research® plus variants (2)	*not applicable*	
Leachables	**Certified absence of additives:** Plasticizer, biocides, slip agents cannot interfere with biological analyses.	> epT.I.P.S.® > ep Dualfilter T.I.P.S.® > ep Dualfilter T.I.P.S.® SealMax®	> Combitips® advanced	

> Light labelling: applicable, dark labelling: recommended
> Family includes all pipette variants (fixed and variable volumes, single- and multi-channel, Move It®)

(1) Variants > 20 µL without metal pistons, except 16- and 24-channel pipettes
(2) Available for Reference 2 and Research plus single-channel pipettes: 1,000 µL (color code: blue); 5 mL (color code: violet); 10 mL (color code: turquoise); available for Reference 2 single-channel pipettes only: 2.5 mL; 2 mL, fixed (color code for both: red).

For more information, technical specifications and article numbers for Eppendorf pipettes, dispensers and pipette tips, visit **www.eppendorf.com/pipettes**.

Interested in learning more about requirements for liquid handling systems and suitable solutions in healthcare applications? Then take a look at White Paper No. 82 "What Really Matters: Manual Liquid Handling Tools for Healthcare Applications".

About Eppendorf

Since 1945, the Eppendorf brand has been synonymous with customer-oriented processes and innovative products, such as laboratory devices and consumables for liquid handling, cell handling and sample handling. Today, Eppendorf and its more than 5,000 employees serve as experts and advisors, using their unique knowledge and experience to support laboratories and research institutions around the world. The foundation of the company's expertise is its focus on its customers. Eppendorf's exchange of ideas with its customers results in comprehensive solutions that in turn become industry standards. Eppendorf will continue on this path in the future, true to the standard set by the company's founders: that of sustainably improving people's living conditions.

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