

## Single use mitogen coated cytometry tubes for lymphocyte stimulation

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In vitro response to mitogen is a laboratory parameter that estimates the function of T-cells in the context of primary or acquired immune deficiency. The in vitro protocols for stimulation and detection of the response were previously focused on radioactivity readings, however for most laboratories today the detection method of choice is flow cytometry. Our aim was to develop a method that would allow stimulation, processing and flow cytometry analysis in a single tube. Mitogens were freeze-dried or vacuum dried inside cytometry tubes and exposed to various stressed conditions (high air humidity and elevated temperatures). The mitogen activity was monitored in short term whole blood cultures with T-cell BlastoFlowEx Kit (Ki-67 expression). Vacuum drying was found more suitable method of production than lyophilisation due to the high hygroscopic nature of lyophilised materials. Vacuum drying produced a thin transparent film that strongly adhered to the plastic. Freeze-drying resulted in white lyophilisation cakes that were loosely attached to the bottom of the tubes and easily displaced. PHA lyophilized without excipients lost 85% of its activity within 3 weeks if stored at 45 °C. PWM and ConA were surprisingly very stable. Air humidity caused shrinkage of lyophilisation cakes and liquefaction of the vacuum dried preparations. However, the activity of excipients stabilized PHA remained unchanged during the 30 days of the stress conditions. Water condensation was the primary destabilizing stress during storage and could only be prevented by protective packaging. Mitogen coated cytometry tubes can be readily adopted by routine immunology laboratory for evaluation of T-cell function.

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