

Room Temperature Stable Unitized Reagents for High Content, High Throughput Flow Cytometry Applications

Authors: Neha Girish¹, Jean-Marc R Busnel², Samee Saif¹, Nathalie Dupas², Tewfik Miloud², Vishakha Mangale¹, Emmaunel Gautherot², Sridhar Ramanathan¹

Affiliation: ¹ Beckman Coulter India Pvt Limited, Bangalore Development Centre, Bangalore, INDIA.
² Beckman Coulter Immunotech, Marseille, FRANCE.

This work was originally presented as a poster at CYTO 2014 Conference, Ft. Lauderdale, Florida, USA.

fast track to success.



Room Temperature Stable Unitized Reagents for High Content, High Throughput Flow Cytometry Applications

ABSTRACT

Development of automated stations for sample processing, use of microplates and multi-spectral fluorescent tags has led to emergence of flow cytometry as a high content, high throughput analysis technique that enables expedient phenotype analysis and rapid epidemiological screenings. Ensuring standardization in sample preparation, reagent handling and analysis methods is vital for large clinical research studies (single or multiple laboratories) that employ high throughput flow cytometry. The use of automated sample processing robotics combined with centralized data analysis has significantly enhanced normalization in high throughput analysis. However, there are inherent limitations brought about by reagent handling which cause significant assay variability. Liquid conjugates have rigid storage requirements, are susceptible to light exposure and manual handling errors while lyophilized reagents have their characteristic drawbacks such as flaking and incomplete reagent re-suspension. Reagents dried using Beckman Coulter's proprietary DuraClone process avoid these problems by providing reagents in unitized format and allow for their storage at ambient temperature. To demonstrate the application of the DuraClone technology to high-throughput flow cytometry, single color antibody fluorophore conjugates and multicolor antibody-fluorophore reagent cocktails were dried down in standard 96-well microtiter plates and their performance characteristics were evaluated. Several subsets of leukocytes were stained with liquid and dry reagents and no significant difference was observed in the staining pattern or intensities. This study demonstrated that the functionality of antibody-fluorochrome is not impacted by the DuraClone process. It also shows that there is no difficulty in re-suspension of the dried reagent into the sample. Temperature stability was evaluated when a plate with the dried-down antibody cocktail was exposed to stress at 40°C and 75±5 % humidity for 3 months. The performance was similar to a dried-down antibody cocktail stored at room temperature for the same period. Intra-plate precision studies were performed on a three color dried cocktail targeting lymphocytes, monocytes and granulocytes across 88 samples using robotic sample processing. There was low variability in cell recruitment and median intensities for all markers, thereby demonstrating the robustness of the technology. This study has therefore demonstrated the potential of the novel DuraClone technology to generate a stable and functional reagent in a high throughput format which can be a powerful tool to ensure standardization in multicolor flow cytometry studies.

MATERIAL AND METHODS

Method 1: Single Conjugates for Stability Study

Bulk concentrated CD3 antibody conjugated to 10 different dyes combinations (Table 1). The 10 conjugates were then formulated to 20 µL per test in a stabilizing buffer in the optimized dose. These single conjugates were dried in nine 96-well round bottom polystyrene plates using the DuraClone technology. The dried plates were sealed using an adhesive film and packed in standard sealed pouches. The control plate was stored at room temperature (20-30°C) and test plates were placed in an environmental chamber controlled at 40±2°C and 75±5 % humidity at pre-scheduled time points (4, 7, 14, 22, 39, 58, 78 and 98 days in stress respectively).

Normal whole blood sample was bulk lysed with VersaLyse Lysing Reagent and 20 µL of this processed sample was stained in triplicate with all conjugates across 9 plates for 15 minutes after 6-8 seconds of vortexing. The stained samples were then transferred to 12x75mm Tarson tubes and 400 µL of 1xPBS was added to increase sample volume before analysis.

Samples were evaluated on Beckman Coulter's Navios^s cytometers equipped with standard configuration of violet, blue and red lasers and with 10 channels. Data were analyzed with Beckman Coulter's Kaluza^s software.

Crosstalk Index was calculated as $\frac{\text{Log SNR (MFI (Secondary))}}{\text{Log SNR (MFI (Primary))}}$ where SNR is the signal to noise ratio, MFI is

Mean Fluorescence intensity, Primary refers to the main tandem channel and secondary refers to the spillover channel.

Table 1: Antibody Conjugates used in the study

Target (Clone)	Fluorophore	Target (Clone)	Fluorophore
CD3 (UCHT1)	FITC	CD3 (UCHT1)	APC-Alexa Fluor* 750
CD3 (UCHT1)	PE	CD3 (UCHT1)	Pacific Blue*
CD3 (UCHT1)	ECD	CD3 (UCHT1)	Krome Orange
CD3 (UCHT1)	PE-Cy 5.5	CD16 (3G8)	FITC
CD3 (UCHT1)	PE-Cy7	CD45 (J33)	PE
CD3 (UCHT1)	APC	CD14 (RM052)	APC
CD3 (UCHT1)	APC-A700		

This application is Research Use Only and not intended for diagnostic purposes even though the Navios was used for the analysis

Method 2: Three color cocktail for Precision Study

Bulk concentrated CD16, CD45 and CD14 antibodies conjugated to FITC, PE and APC respectively (Table 1). The mixed 3 color cocktail was then formulated to 20 μL per test in a stabilizing buffer in the optimized dose. 96 tests of this cocktail were dried in a single 96-well round bottom polystyrene plate using the DuraClone technology.

Using Beckman Coulter's BioMek automated robotic sample handler, 20 μL of whole blood was added to 88 wells across the plate and stained for 15 minutes following 10 seconds of automated mixing. 180 μL Optilyse C Lysing solution was added to the wells and allowed to incubate for 15 minutes.

Samples were evaluated on Beckman Coulter's FC500 cytometer equipped with a Multi Plate loader compatible with the plate and data was analyzed using Kaluza Software.

RESULTS

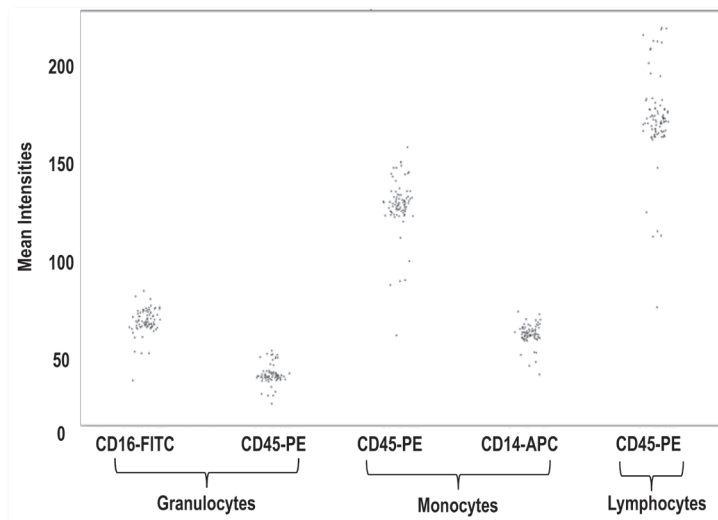


Figure 1: MFI of 88 repeats of 1 normal whole blood donor on 3 color cocktail targeting granulocyte, monocyte and lymphocyte subsets. The CV of CD16⁺ granulocytes and CD14⁺ Monocytes is less than 6% and that of CD45⁺ Lymphocytes is less than 15 %.

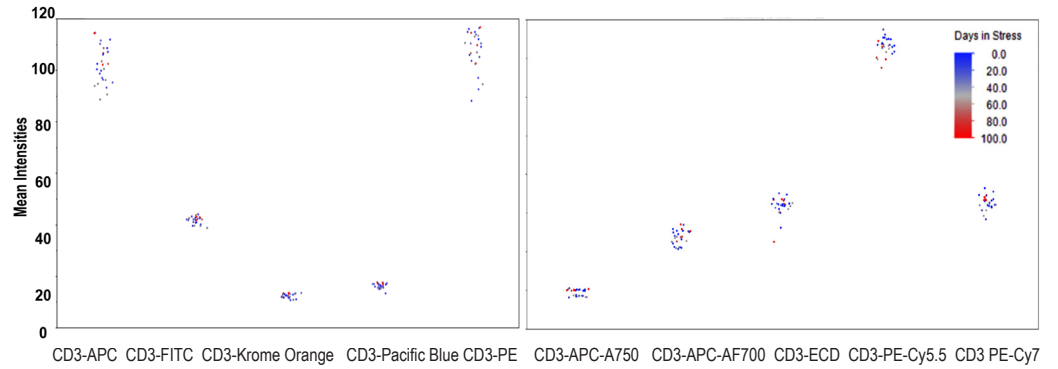


Figure 2: Median Intensities (MFI) of non-Tandem conjugates and Tandem Conjugates over 98 days in stress. All conjugates were stable across 98 days in stress with no drop in mean Intensities.

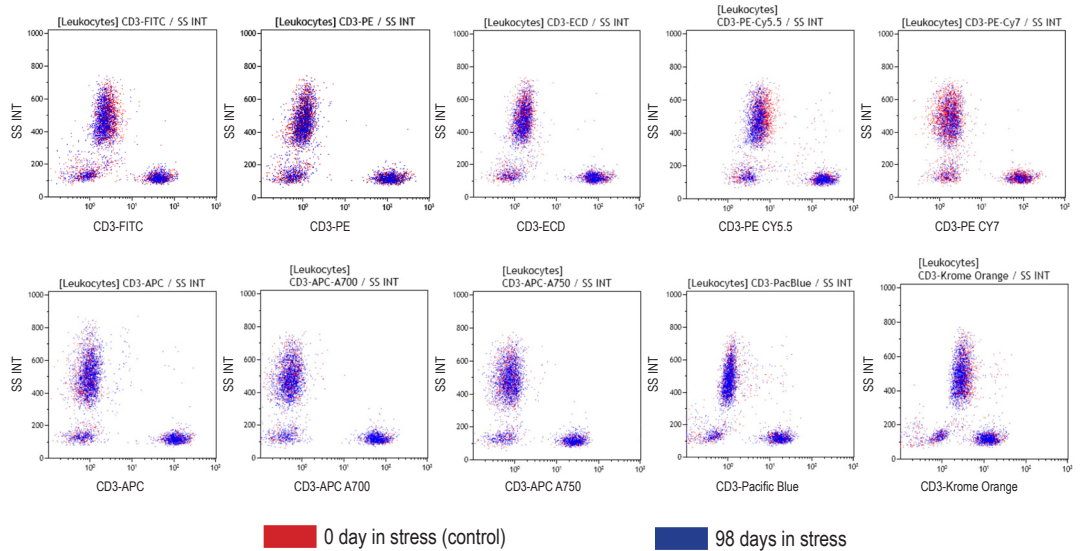


Figure 3: Overlays of single CD3 conjugates stained with dried reagent stressed for 98 days and control reagent for all dye combinations

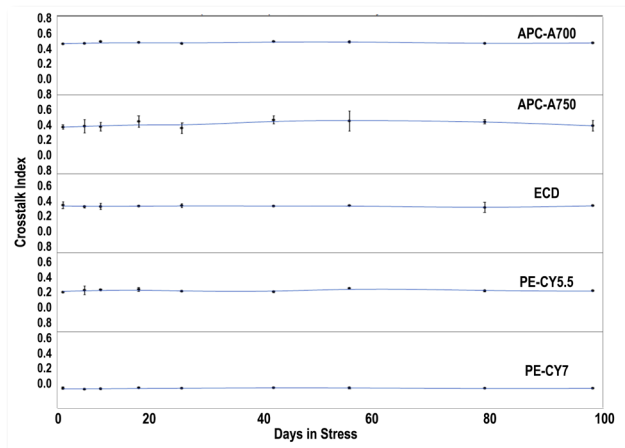


Figure 4: Crosstalk index of Tandem conjugates on their parent channels over increasing days at Stress. At each time point, the mean and standard deviation of replicates of 3 data points is represented. Crosstalk of all tandems remained constant over 98 days in stress.

SUMMARY

- Stability of dried reagent conjugates across several dye combinations for up to 3 months in stress conditions has been successfully established
- No degradation of tandem dyes and consistent spillover across the stability period
- The Intra-plate precision of multicolor reagent shows consistent staining and recruitment of the 3 main leucocyte subsets namely lymphocytes, monocytes and granulocytes indicating suitability of DuraClone reagents for multicolor flow cytometry
- This study has successfully demonstrated the DuraClone plate technology to be a robust and stable tool for high throughput, high content flow cytometry

Research Use Only, Not Intended for Diagnostic Purposes

§ Navios is CE marked for 10-color in-vitro diagnostic use. In the U.S., Navios is intended for use as an in-vitro diagnostic device for immunophenotyping with Navios tetra software and CYTOSTAT tetraCHROME reagents. All other uses are for research use only.

** Alexa Fluor and Pacific Blue are trademarks of Molecular Probes, Inc.*

Biomek, DuraClone, FC500, Kaluza, Krome Orange, Navios, OptiLyse, and Versalyse are trademarks of Beckman Coulter, Inc. Beckman Coulter and the stylized logo are trademarks of Beckman Coulter, Inc and are registered in the USPTO.