

# Application Information Bulletin: DuraClone and Lysis

## DuraClone Custom Design Service Reagents: Recommendations on Lysis buffers and Protocols

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# DuraClone Custom Design Service Reagents: Recommendations on Lysis buffers and Protocols

## BACKGROUND

The DuraClone line of reagents introduced by Beckman Coulter are dried reagents that are in a ready-to-use format. These reagents are shelf-stable at room temperature, simplify workflow, minimize hands-on time, and have been shown to deliver the same consistent performance as liquid reagents. Beckman Coulter has expanded its Custom Design Services (CDS) portfolio to these reagents, offering multicolor cocktails in a dry, temperature-stable format.

The reagents, which are a mixture of antibody conjugates, are dried using a propriety process in the presence of a stabilizing buffer containing additives like proteins and carbohydrates. The presence of these additives in the dried reagent may cause incomplete lysis of stained whole blood compared to a liquid reagent. An increase in the unlysed red blood cells is observed in the forward and side scatter channels which affects the number of leukocytes acquired in the total number of events compared to a liquid reagent and may affect the percentage of gated cells by impairing the purity of the lymphocyte or monocyte populations on scatter based gating

One way to reduce this effect of DuraClone additives on red cells lysis is to dilute these additives by increasing the volume of lysis buffer or increase the time of incubation of the buffer to enable sufficient time for red cell lysis.

In this paper we compiled the results of our investigations and describe modifications of the standard protocols for Beckman Coulter lysing reagents. Using these modified protocols, equivalent lysing results can be obtained for staining with dry and liquid conjugate mixes.

## MATERIAL AND METHODS

### Lysis Buffers evaluated from the Beckman Coulter Catalog

For extracellular applications, the following buffers were evaluated:

1. VersaLyse Lysing solution, PN A09777
2. OptiLyse C Lysing solution, PN A11895
3. IOTest 3 Lysing solution , PN IM3514

For intracellular applications, the following buffers were evaluated

1. IntraPrep Permeabilization Reagent , PN IM2388
2. PerFix-nc Kit, PN B10825

### Evaluation of Lysis Efficiency

All of the data generated on the DuraClone reagent were compared to a control reagent, which was a liquid reagent without any additives.

Extracellular analysis:

The following panel was used for extracellular analysis of the stain- lyse -wash and no wash protocols:

Panel 1: CD16b-FITC / CD14-APC / CD45-Krome Orange

Panel 2 : CD16-FITC/ CD14-PC7/CD45-Krome Orange

The following panel was used for extracellular analysis of bulk lyse –wash- stain protocols and pre-lyse -wash:

Panel 3: Kappa-FITC / Lambda-PE / CD19-ECD / CD14-PC7 / IgD-APC / CD16-APC-Alexa Fluor\* 750/ IgM-Pacific Blue\* / CD45-Krome Orange

The following panel was used for intracellular analysis:

Panel 4 : MPO-FITC / CD79a-PE / CD3-ECD / CD14-Pacific Blue\* / CD45-Krome Orange

1. Lymphocyte purity: Lysis efficiency was evaluated by calculating the purity of the lymphocyte population in the expected region on forward and side scatter. The recovery of the lymphocytes was set to 90% for all data files to ensure standardization of the blood samples collected. The recovery of lymphocytes to 90% is done manually by adjusting the lymphocyte gate on the forward scatter versus side scatter to 90% with a parent gate of LowSSC+CD45+CD14- events to ensure “true” lymphocytes are analyzed. Lymphocyte purity of >80% was acceptable
2. Median fluorescent intensity (MFI): The intensities of the respective cell populations were compared to the intensities of the respective cell populations in the control. The median intensities of populations > 75% of Control was acceptable.
3. Cell recruitment: Cell recruitment is defined as the % gated population in the global parent population (Example % CD3+ cells of CD45+ lymphocyte cells)The gated cell population in the dried reagent was compared to the gated cell population in the control.
4. Cell count: The cell count was evaluated against the cell count from the control in the following protocols; Extracellular Stain-Lyse wash and no wash approach using VersaLyse 2mL, IO Test 3 Lysis, Opti C lysis.

## RESULTS AND RECOMMENDATIONS:

The consolidated data from evaluation of lysis buffers on DuraClone reagents is shown in the table below. The lysis protocol change, if any, is mentioned in the sample protocol. The Lysis buffers and their protocol are rated as per the following color codes:

	<b>Not recommended</b>	Not recommended as the lymphocyte purity is lower than 80% or/and MFI of Duraclone is > 25% lower than the lymphocyte MFI in the liquid control or cell count and recruitment is not matching with that of the control
	<b>Neutral</b>	Lymphocyte purity is <80% but MFI and cell count is equivalent to that of the liquid control
	<b>Recommended</b>	Equivalent to liquid in all metrics (MFI, % recruitment). Equivalent to liquid in cell counts in extracellular staining protocols for Stain-Lyse-wash and no wash

Sample Protocol	VersaLyse 1 mL, +10 min.		VersaLyse 2 mL, +10 min.		IOTest 3 2 mL, +15 min.		IOTest 3 2 mL, +30 min.		OptiLyse C 1 mL, 10 min.	
	FSC gate	CD45 gate	FSC gate	CD45 gate	FSC gate	CD45 gate	FSC gate	CD45 gate	FSC gate	CD45 gate
Stain-Lyse-No wash										
Stain-Lyse-Wash										
Stain-Lyse-Pre-wash; Wash									* Low negative to positive discrimination	
Bulk Lyse-Wash-Stain									* Fixative in Lyse => non-specific staining of IgD...	

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