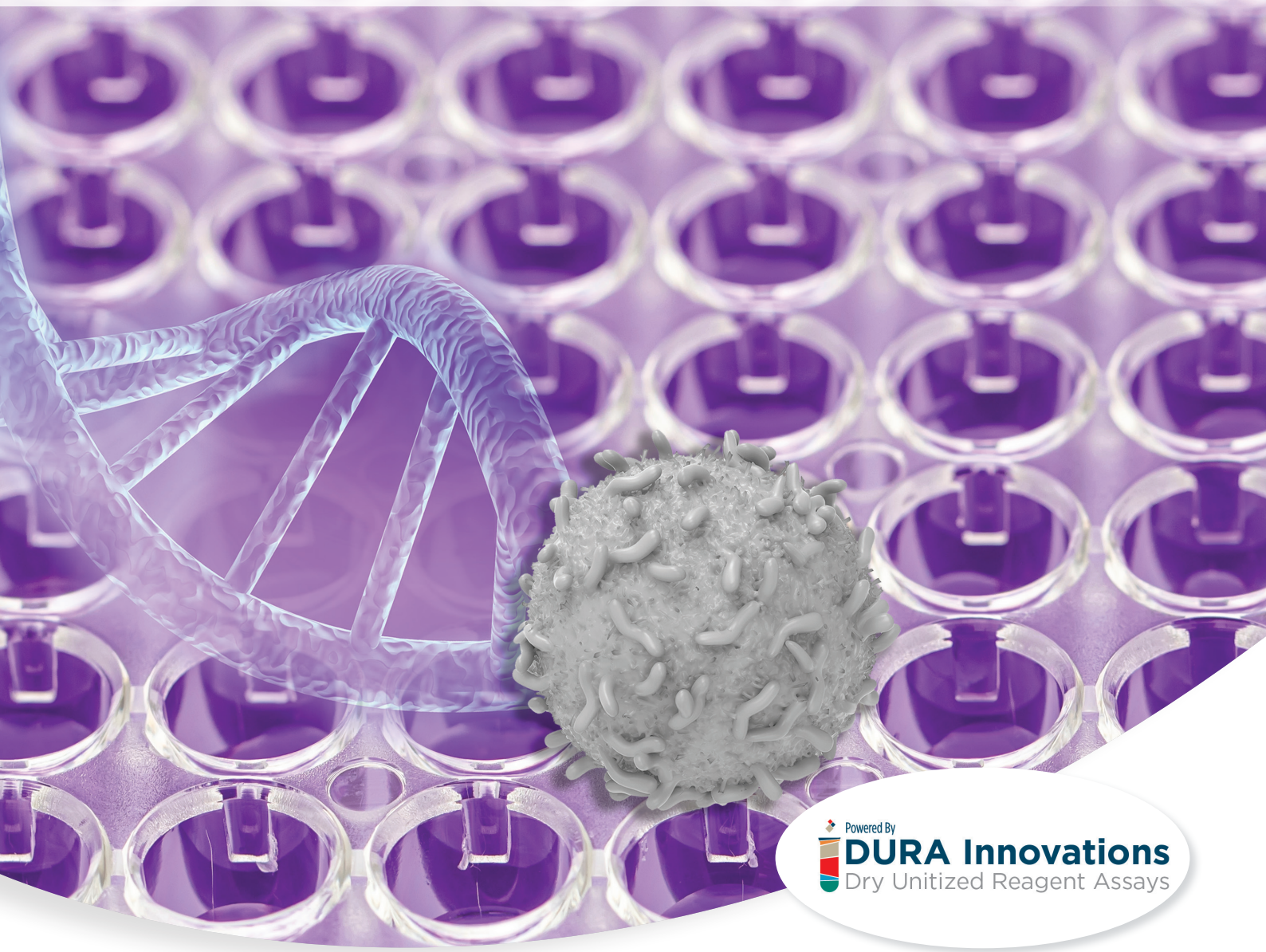
The DURAClone RE CTC and PerFix CTC reagent system

THE FLEXIBILITY TO INVESTIGATE HETEROGENEITY



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Dry Unitized Reagent Assays

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Life Sciences

THE FLEXIBILITY TO INVESTIGATE HETEROGENEITY

As an open platform technology, flow cytometry can uniquely accommodate inter- and intratumoral heterogeneity of circulating tumor cells (CTCs) as opposed to many purpose-built platforms that rely on rigid morphological, immunological and/or physical features. For example:

Biological feature/challenge	Analytical Feature/challenge	Adaptation by flow cytometry
Phenotypic instability	Loss of epithelial markers (epithelial-mesenchymal transformation, EMT)	• Include markers for transformed cells (e.g., Vimentin) • Record all cells • Identify, count and characterize all CTCs
Morphological diversity	Varying cell size and intracellular complexity	• Record all cells, • Differentiate morphology of all CTCs by analysis of scatter characteristics
Formation of cell clusters	Physical association of tumor and non-tumor cells	• Add markers for hematopoietic cells • Record all cells, including clusters • Reveal cluster composition involving all CTCs by analysis of marker patterns
Different tumor entities	Tumor type-specific marker expression	• Match tumor type with specific markers • Record all cells • Identify, count and characterize all CTCs
Sensitivity of nucleic acids to fixation/permeabilization	Preservation of high genome integrity in isolated single cells	• Mild fixation/permeabilization chemistry • Direct centric deposition into wells of 96/384 plates by cell sorting

Specific challenges in CTC analysis and corresponding flow cytometric methodology

DURAClone RE CTC AND PerFix CTC

The DURAClone RE CTC and PerFix CTC reagent system is the first ready-to-use solution for flow cytometry that enables identification and isolation of human circulating tumor cells from whole blood.

This new system delivers:

- High specificity due to an expert-proven marker combination and staining protocol
- High sensitivity through compatibility with up to 5 mL of whole blood
- Easy data acquisition without the need for spillover correction
- Less risk of error and variability by eliminating antibody pipetting and wash steps (only 1 spin-down step required)
- High yield of intact single-cell DNA through mild preparation chemistry (see also following pages)

DURAClone RE CTC Tube (C75112, 15 tests, RUO)

Product	PB	KrO	FITC	PE	ECD	PC5.5	PC7	APC	AF647	AF700	APC-AF700	APC-AF750	Quality Standard
RE CTC Tube C75112 (15 tests, RUO)	DAPI	-	Cyto-keratin	-	-	-	CD45	EpCAM	-	-	-	-	ISO 9001-2015

PB: Pacific Blue*
KrO: Krome Orange

AF647: Alexa Fluor* 647
AF700: Alexa Fluor* 700

APC-AF700: APC-Alexa Fluor* 700
APC-AF750: APC-Alexa Fluor* 750

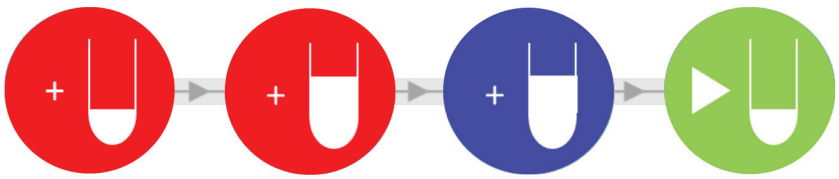
PerFix CTC (C75118, 15 tests, RUO)

PerFix CTC Buffer 1	PerFix CTC Buffer 2	PerFix CTC Buffer 3
Fixative Reagent 1 vial, 1.9 mL 100 µL/test	Permeabilizing Reagent 4 vials, 25 mL each 6 mL/test	Final 10X Solution 1 vial, 26.3 mL, 10X conc.) 100 µL/test

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

NON-EXPERTS IN FLOW CYTOMETRY WELCOME!

The PerFix CTC staining protocol is designed to **enrich cells** from large volumes of whole blood while **reducing the risk of cell loss and operator-dependent variability** associated with repeated washing steps. The unitized format of DURAClone RE CTC, provided as a dry room temperature-stable layer at the bottom of the reaction tube, **eliminates pipetting errors and variability** associated with human liquid handling and reagent aging effects.

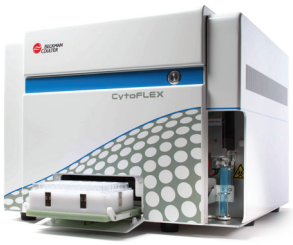


Fixation of 1-5 mL whole blood sample (PerFix CTC buffer 1)

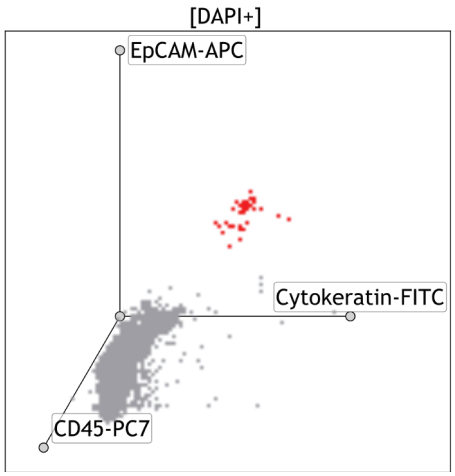
Permeabilization & lysis (PerFix CTC buffer 2)

Surface & intracellular staining (DURAClone RE CTC)

Spin down, resuspend & acquire (PerFix CTC buffer 3)



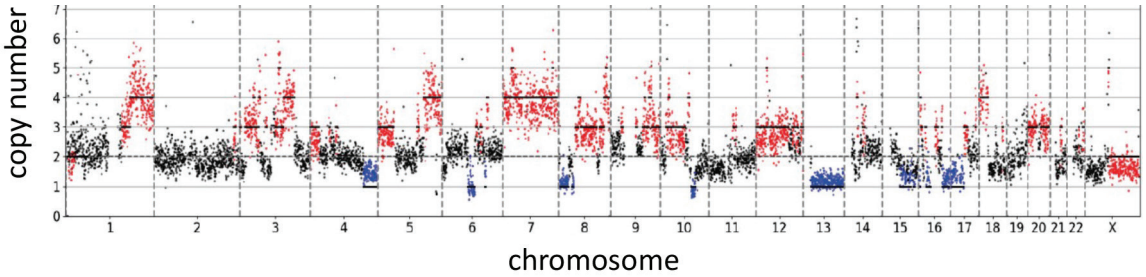
Ideally suited for CytoFLEX Flow Cytometers



A **carefully selected** set of **markers** (DAPI, CD45, EpCAM, Cytokeratin), together with **minimal spillover** between the respective fluorescent labels, ensures **unambiguous discrimination of CTCs** from excessively abundant leukocytes.

CONFIDENCE IN IDENTITY AND INTEGRITY

Using the DURAClone RE CTC and PerFix CTC system, you can be confident in isolating CTCs as demonstrated by the typical occurrence of chromosome copy number variations in an isolated target cell.



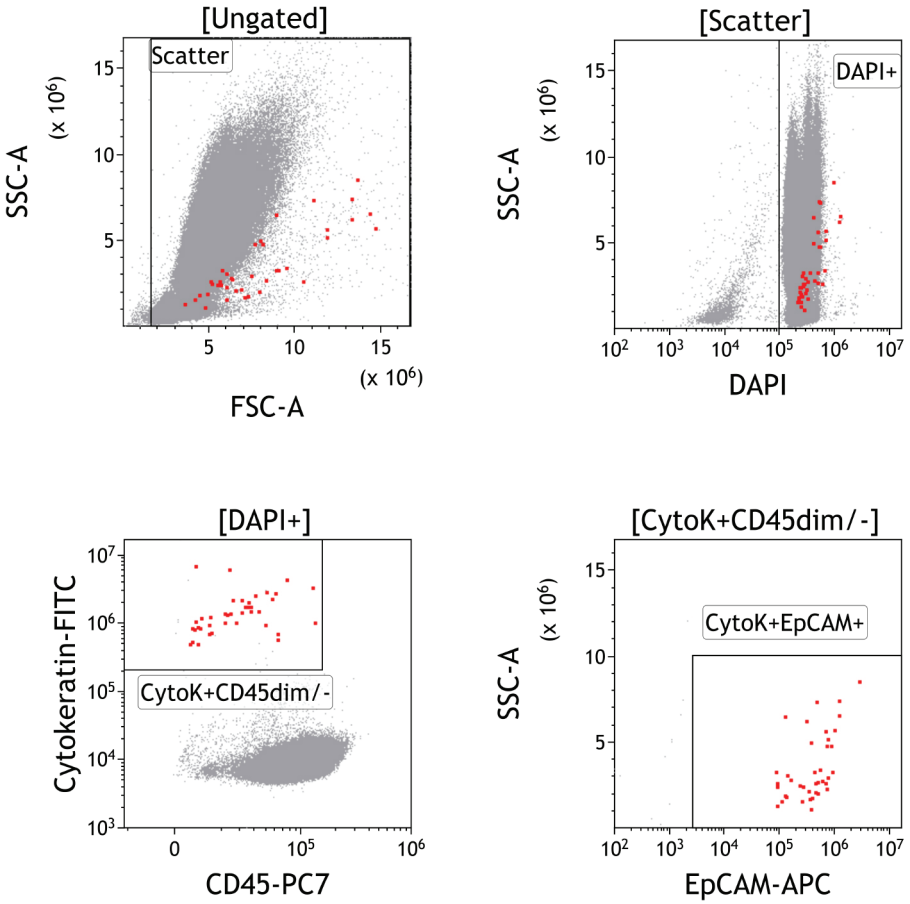
In the isolated CTCs, high DNA quality is associated with a genome integrity index (GII) of 2 or higher.[1] The table below shows the yield of cells from 3 samples with a GII ≥2.

	CTC (EpCAM+Cytokeratin+)	
Sample	Isolated Cells	Thereof with GII* ≥2
1	1	1
2	3	1
3	10	9

* genome integrity index

[1] Polzer B et al. EMBO molecular medicine vol. 6,11 (2014): 1371-86.

DATA EXAMPLE



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RE CTC Tube C75112 (15 tests, RUO)	DAPI	-	Cyto-keratin	-	-	-	CD45	EpCAM	-	-	-	-	ISO 9001-2015

The DURAClone RE CTC tube is designed to identify CTCs by their expression of EpCAM and Cytokeratin, allowing for heterogeneity in cell size and flexible addition of further markers.

REAGENTS FOR FLEXIBLE ADAPTATION

Specifcity	Target entity	Compatible reagent available from Beckman Coulter
CD15	CTC cluster-associated neutrophils[2]	• PE (IM1954U) • Krome Orange (B01176)
CD66b	CTC cluster-associated neutrophils[2]	• APC-AF750 (B08756)
CD24	Stem CTCs in breast cancer[3]	• PE (IM1428U, B92425) • ECD (B12699) • PC5.5 (B23133) • APC-AF750 (B10738)
CD44	Stem CTCs in breast cancer[3]	• PE (A32537) • APC-AF750 (B30637)
CD41	Carcinoma-associated fibroblasts[4]	• PE (IM1416U, A07781) • ECD (6607117)
CD61	Carcinoma-associated fibroblasts[4]	• PE (IM3605) • PC5.5 (B21172)
Vimentin	CTCs under epithelial-mesenchymal transition[5]	-

[2] Szczerba BM et al. Nature vol. 566,7745 (2019): 553-557.
[3] Savelieva OE et al. International journal of molecular sciences vol. 21,8 2780. 16 Apr. 2020.
[4] Duda DG et al. PNAS the United States of America vol. 107,50 (2010): 21677-82.
[5] Wu S al. PloS one vol. 10,4 e0123976. 24 Apr. 2015.

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