IsoFlux™ CTC Enrichment Kits
Validation Data and Intended Use

Description

The IsoFlux System utilizes several different kits for isolation of CTCs and other rare cell types from biological samples (whole blood, fractionated blood, ascites, etc.). Each kit incorporates eight (8) IsoFlux microfluidic cartridges for high-sensitivity immuno-magnetic enrichment and the reagents necessary to perform positive cell enrichment for eight samples. The included bead reagents are either pre-conjugated with anti-EpCAM antibodies, or ready to be conjugated with antibodies included in the kit. Available antibodies include both epithelial and mesenchymal markers, depending on the desired cell type.

The kits described are intended for use with the IsoFlux System. For research use only.

Fig. 1. The IsoFlux reagent kit.

CTC ENRICHMENT KITS

KIT NAME	PART NUMBER	DESCRIPTION
CTC Enrichment Kit	910-0091	This kit was the first kit to be commercially available and validated for high sensitivity recovery of CTCs. Recovery based on EpCAM antigen presence.
Enhanced CTC Kit	910-0108	Validated for most applications and recommended for the high purity NGS workflow. Recovery based on EpCAM and EGFR.
EMT Enrichment Kit	910-0106	Validated to recover both epithelial and mesenchymal cell types, this is the most comprehensive set of recovery markers. Four different markers are used, including two mesenchymal marker plus EpCAM and EGFR.
Rare Cell Kit, IgG	910-0092	The RCE kit was designed with maximum flexibility in mind. The magnetic bead surface is coated with pan-mouse IgG molecules, allowing the user to couple their own antibodies to the beads for targeted cell capture.

SPECIFICATIONS

Number of samples: Each kit processes 8 individual samples
Shelf life: 6 months

VALIDATION DATA

Both clinical and analytical data have been generated to validate the performance of the enrichment kits described above. Clinical validation has been presented as part of published reports, a subset of which can

be found in Liquidbio's publication library. This document will present analytical validation of the kit performance. To validate recovery of tumor-derived cells, target cells are spiked into whole blood (7.5mL, EDTA purple top tubes), recovered using the IsoFlux system, and counted using published IsoFlux enumeration protocols.

1. Immunomagnetic isolation efficiency depends on antigen expression levels

An important aspect of immunomagnetic separation that is often overlooked is the dependence of recovery efficiency on antigen expression, in most cases EpCAM expression. Patient tumor samples and tumor cell lines both show significant variability of greater than 10x in antigen expression – that is the case within CTC populations as well (Punnoose et al.).
The reported variability in EpCAM expression for both cell lines and tumor tissue as reported by several groups was described in our recent white paper.

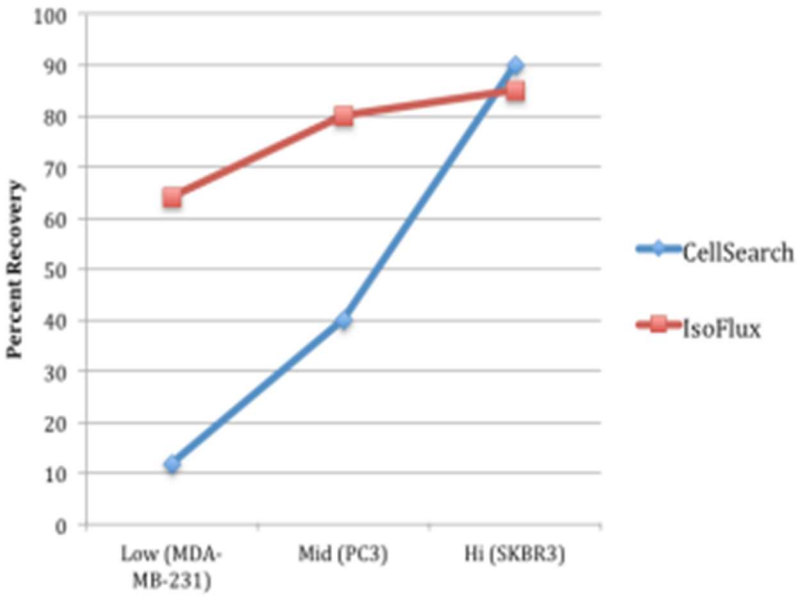

Fig 2. Recovery using the EpCAM-based CTC Enrichment Kit compared to CellSearch EpCAM recovery. IsoFlux cell line recovery was measured by labeling target cells before spiking into blood, making the results independent of coun0ng bias. PC3 recovery for both platforms was measured using matched samples and protocols. CellSearch MDA-MB-231 and SKBR3 data is reported in literature using similar spiking experiments

A great majority of the analytical data characterizing EpCAM-based CTC isolation has been obtained on the CellSearch system – until recently, this was the only commercially available immunomagnetic separation instrument.
That system has been shown to perform well (recovery > 80%) for cell lines that are high EpCAM expressers like SKBR3. For mid to low expressers, percent recovery measured using cell lines is significantly lower.
Analyses of CellSearch recovery data across cell lines with varying levels of EpCAM expression consistently report lower recovery for cell lines that are in the mid to low expresser category (Sieuwertz et al., Punnoose et al.). Low analytical recovery from a number of cell lines using the CellSearch system has led a number of groups to conclude that EpCAM-based CTC isolation is a low-sensitivity technique that misses a significant percentage of CTCs present in patient samples (Farace et al., Sieuwertz et al., Punnoose et al.). Low CTC recovery makes it difficult to effectively address diseases like lung cancer, for example.
IsoFlux data and data from several sources demonstrate that it is possible to efficiently recover cells of much lower EpCAM expression by using immunomagnetic separation systems with much higher sensitivity (Fig. 1). This has been shown by both data from the IsoFlux system (Alva et al., Harb et al.) and data by other research groups utilizing microfluidic separation technology (Ozkumur et al.).

2. The addition of EGFR as a selection marker improves recovery of very low to no EpCAM cell lines

For certain indications, it has been well documented that the relative EpCAM expression is low, resulting in lower cell numbers and percent patients that are determined to be CTC positive, even when presenting with advanced disease. A good example is lung cancer, where the fraction of patients with CTCs detected by the FDA approved CellSearch system is only about 15-25% (Tanaka et al.). Cell lines like A549, a lung carcinoma cell line that is described by many to be EpCAM negative, are good models for recovery optimization. We believe that this cell line does indeed express EpCAM, albeit at very low levels that are not easily detected by imaging. In controlled cell recovery experiments (pre-labeled A549 cells) we obtained recovery numbers averaging 40% with high sensitivity EpCAM alone, but this increases dramatically to 73% when EGFR-functionalized bead reagents are added (Fig. 3).

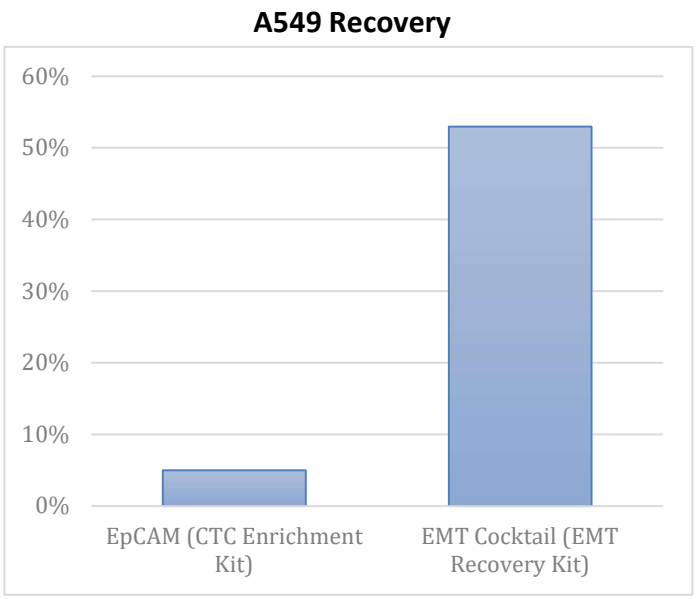

Fig 3. Enhanced recovery of a lung cancer cell line when using EGFR based recovery. IsoFlux cell line recovery was measured by incubating a set number of A549 cells with bead reagent and recovery in the IsoFlux instrument. Significant gains are shown for this lung carcinoma line.

3. The addition of mesenchymal markers enables recovery of cell lines that are known to be negative for epithelial markers and have undergone EMT.

Another area where positive selection markers have been deficient is in the recovery of CTCs undergoing the epithelial-to-mesenchymal transition (EMT). As with epithelial cells, mesenchymal cells express protein markers that are known not to be expressed in white blood cells (WBCs). Consequently, the same principles can be used to recover cells that are completely negative for epithelial markers. One such cell line is Hs578T. The is a cell line that was shown to have no recovery using CellSearch (Sieuwerts et al.) and for which IsoFlux experiments using EpCAM have only had very modest recovery, below 5%. The line has robust expression of known EMT markers, but no epithelial marker expression (Sieuwerts et al.). Liquidbio has developed a cocktail of enrichment markers

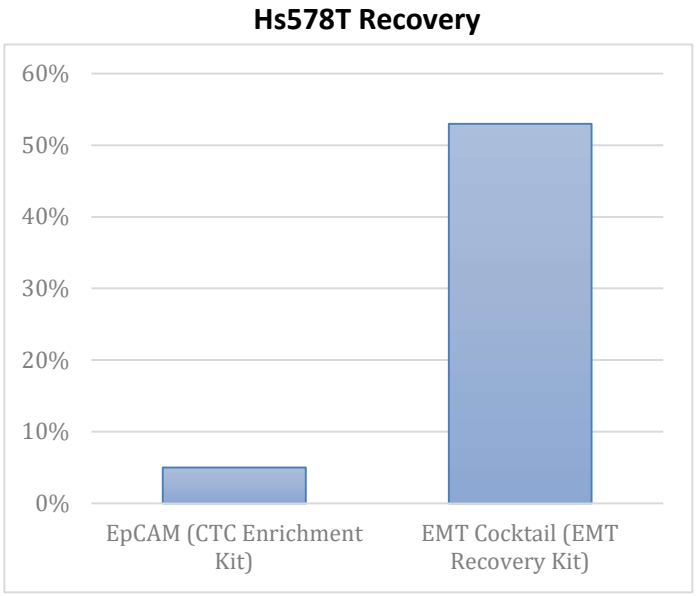

Fig 4. Cells with mesenchymal characteristics are recovered using the IsoFlux EMT cocktail. When spiked into whole blood and recovered using the IsoFlux instrument, mesenchymal tumor-derived cell line Hs578T is not recovered by EpCAM, but robustly recovered when using an antibody cocktail in the IsoFlux EMT Recovery Kit.

that demonstrate robust recovery for this cell line using the EMT Enrichment Kit (Fig. 4). The EMT Kit has also shown improved recovery of the breast cancer cell line MDA-MB-231, which is a mesenchymal-like line that also shows low epithelial marker expression. This kit is recommended for experiments where recovery of both mesenchymal and epithelial CTCs is desired. Antibodies 3/4 and 4/4 in the kit are specific to EMT cells, and they can be used alone to separate only cells expressing these markers; however, we believe that there is a significant overlap between these populations, or a class of CTCs in circulation that express both marker types; consequently, separating a 'mesenchymal only' set of cells is likely to be difficult in practice.

CONCLUSIONS

Both clinical and analytical data have been generated to validate the performance of the enrichment kits that use positive selection to recover tumor cells from blood, spanning the epithelial to mesenchymal transition spectrum. This technical note contains a description of the kit offerings and supporting analytical cell recovery data from spikes into whole blood. Further evidence of kit performance when using clinical samples can be found in published data from IsoFlux customers and collaborators and is available in Liquidbio's technical library.

REFERENCES

Alva et al., J Urology (2015) 194: 1-9
Danila et al., Clin Cancer Res (2011) 17: 3903-3912
Harb et al., Transl. Onc. (2013) 6(5): 528-538
Ozkumur et al., Science Transl Med (2013) 5: 1789ra47
Punnoose et al., PLOS One, (2010) 5(9): e12517
Sieuwerts et al., J Natl Cancer Inst (2009) 101: 61–66
Tanaka et al., Clin Cancer Res (2009) 15(22): 6980-6986

ORDERING INFORMATION

Purchase orders can be sent to orders@liquidbio.co

www.liquidbio.co

San Francisco, CA USA