

## CFC Assays for Characterization of Compound Function (Working Group 3)

The assays shown below have been developed by the CFC for potential use in drug discovery. Numerous other valuable assays exist within the CF scientific community; a goal of the CFC is to expand upon the list shown below.

Purpose	Assay	CFC and collaborators	Level of Specialization required	Examples
Identification of wild type or mutant CFTR chloride transport by short circuit current measurement (Isc).	Ussing chamber analysis	Sorscher Rowe Others	4 Electrophysiology expertise, Ussing chamber setups.	Corrector compound effects on $\Delta$ F508 CFTR activity. (Rowe)
Testing conformational stability of proteins.	<i>In vitro</i> and in cell translation and limited proteolysis	Braakman Lukacs	3-4	Examination of conformational stability of wt and $\Delta$ F508 NBD1, influence of CFTR corrector compounds on proteolysis.
Detection of CFTR domain interactions.	ELISA	Lukacs Sorscher Others	2	Binding of isolated WT and $\Delta$ F508-NBD1-1S to loop IV and core NBD2 peptides of CFTR in the presence of CFTR correctors (Lukacs).
Measuring correct conformational folding of CFTR domains.	CDP assay	Thomas	4 Fine tuning of CFTR unfolding and partitioning across size exclusion membrane	Effects of the CFTR modulator panel on re-folding of wild type and $\Delta$ F508 NBD1.
Detection of wild type or $\Delta$ F508 CFTR at the cell surface.	Cell based ELISA	Lukacs Sorscher	2	Effects of CFTR correctors on normalized cell surface density of $\Delta$ F508 CFTR-3HA in IB3 cells and Hela cells (Lukacs).
Detection of changes in protein folding by temperature shift.	Differential scanning fluorimetry	Lukacs	3	Detection of unfolding temperature of isolated wt and $\Delta$ F508 NBD1 in the presence of CFTR correctors.

Level of Specialization: 1 – Readily adaptable to a conventionally equipped laboratory,  
4 – Considerable optimization, specialized equipment or technical expertise required

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Detection of cell surface expression of CFTR.	Biotinylation and Immunoprecipitation	Sorscher Collawn Lukacs Others	2-3	Corrector effects on the surface density of $\Delta F508$ in CFBE41o <sup>-</sup> cells (Collawn).
Monitor wild type or mutant CFTR at the cell surface with halide sensitive fluorescence dyes or iodide efflux.	SPQ assay Iodide efflux	Frizzell Pollard Balch Sorscher Others	3 Fluorescence microscopy equipment and monitoring of living cells	Numerous reports in $\Delta F508$ CFTR literature
Evaluate $\Delta F508$ processing in primary human airway epithelial cells.	Function and membrane biochemistry	Frizzell	4	Compound corrector analysis from modulator library
Co-translational folding assay	FRET based	Skach	4	Evaluate nascent chain folding on ribosome

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