



## Fluorescence-based Thermal Shift Assay

The fluorescence-based thermal shift assay is a recently adapted means to perform affinity screening based on a well-known theory.

### Theory behind the assay:

Ligand binding to a target protein can stabilize a protein's native state, as shown in the increase of the bound protein's melting temperature. The midpoint of the melting curve of a protein will increase in the presence of ligands that bind more tightly to the native state than the unfolded state.

### How does the assay work?

The assay takes advantage of an environmentally sensitive fluorescence dye, such as Sypro Orange, and follows its signal changes while the protein undergoes thermal unfolding. When Sypro Orange is added to a properly folded protein solution, it is exposed in an aqueous environment and its fluorescence signal quenched. As the temperature rises, the protein undergoes thermal unfolding and exposes its hydrophobic core region. Sypro Orange then binds to the hydrophobic regions and becomes unquenched. This will result in the increase of fluorescence signal of Sypro Orange.

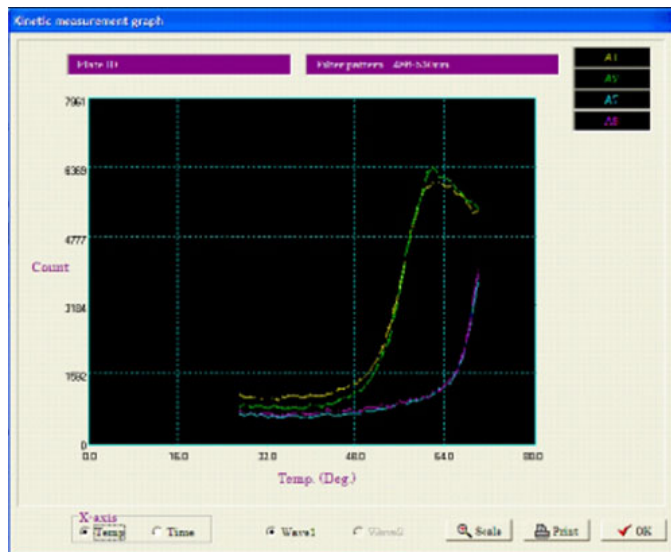


Figure 1: Ligand binding in wells A7, A8 increases the DT . m  
Shown in a temperature vs. fluorescence signal format.

### Application:

Currently, there is a rapid growing interest in the thermal shift assay due to the following reasons:

1. There have always been many interests in screening compound libraries for ligands of target proteins whose function or structure hasn't been identified yet.
2. The newly found application of environmental sensitive dye, such as Sypro Orange, whose signal changes as its bound protein undergoes structural changes.
3. The availability of the *FluoDia T70 High Temperature Fluorescence Microplate Reader*, to perform this type of assay.

The fluorescence-based thermal shift assay is now widely used as a method to identify inhibitors of target proteins without knowing the protein's function and its binding site. The ligand-binding affinity of any potential inhibitor can be assessed from the shift of the unfolding temperature (DT) obtained in the presence vs absence of the potential inhibitor.

### Benefits of the assay

1. It allows for identification of active compounds that bind to the folded form of a target protein *without the need for any knowledge of the binding site or the function of the protein.*
2. It also enables identification of molecules that bind to the unfolded protein. Since molecules that bind to the unfolded protein tend to be protein destabilizers, to exclude them from become potential leads early on can *help reduce the cost of drug discovery.*
3. It allows monitoring protein-melting curves in the *96-well or 384-well plates*, which greatly lower the volume and requirement of the proteins.

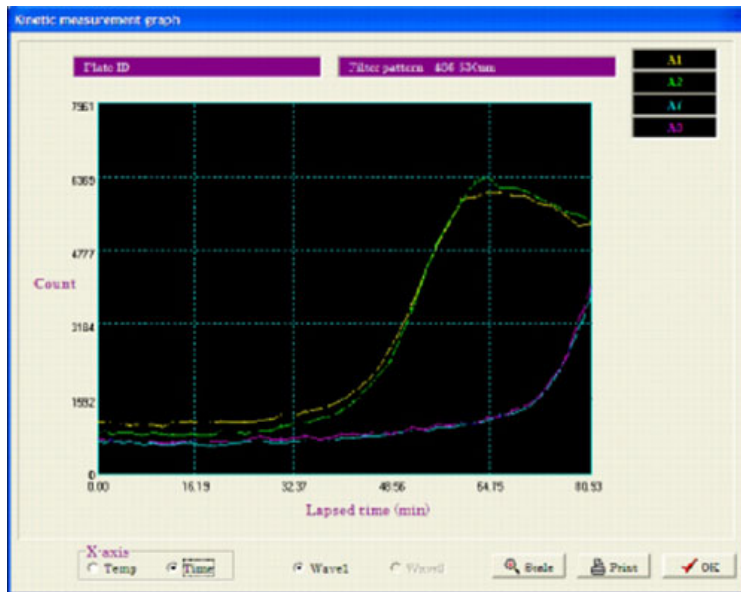


Figure 2: Ligand binding observed in wells A7, A8.  
Shown as Time vs. Fluorescence counts.

### Use of FluoDia T70 for fluorescence based thermal shift assay

So far the FluoDia T70 is the only fluorescence plate reader that is designed to perform this assay. Its unique designed heating plates allow very accurate temperature control up to 75°C with accuracy  $\pm 0.3^{\circ}\text{C}$  and homogeneity  $\pm 0.3^{\circ}\text{C}$  across the plate. Its unmatched temperature control capability, plus its high sensitivity and reproducibility, wide dynamic range (up to 7 decades, best available in market) make it the perfect instrument for this assay. Its user-friendly software allows researchers to monitor the signal changes in a graphic format as proteins undergo thermal unfolding.

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