Sample request form has to be filled out by scientists that want to participate in the demo. The number of measurements per demo is limited, to reserve a place please send back the completed sample request form 1 week prior to the demo. Please register yourself and your samples by sending back the completed form.

For any further question, please contact: teresia.hallstroem@nanotemper.de, phone: +49 89 452289539.

For sample preparation, please consider: all assays are setup in a way that one molecule is fluorescently labeled (**Fluor**.) and a non-fluorescent molecule (**titrant**) is titrated. The titration starts at 20 x concentration above the expected dissociation constant (*Kd*). NanoTemper provides labeling kits to label proteins with a MW of >10 kDa within 1 hour.

**The following material is needed:**

* **50 - 100 µl, 5 - 20 µM of the protein to be labeled** *(in any buffer, > 50 mM Imidazol or > 10 % Glycerol, or DTT, or β-ME should to be avoided)*
* **50 µl of the binding partner with a concentration of at least 20x above the expected *Kd*** *(it is best to provide the highest stock concentration)*

***PLEASE NOTE****: check solubility of e.g. small molecule compound at high concentrations & make sure that e.g. DMSO, EtOH concentration does not exceed the tolerance of e.g. your protein!*

* **50 ml of your interaction buffer** *(Please provide buffer recipe)*

**The following information is required:**

* Information about PI / groups providing the samples
* Information and e-mail contact of the experimenter
* Information about the method that has been used to characterize your type of interaction previously (ITC, SPR, FA, FCS, etc)
* Information about the expected affinity
* Information about the type of molecule (small molecule, protein, DNA, RNA etc), concentration and molecular weight
* Information if the molecule is already labeled (which dye?) or has to be labeled
* Add information about the fluorescent binding partner (kept at constant concentration) and the non-fluorescent binding partner (titrant)
* Please let us know if you have any specific questions regarding this measurement (assay optimization or troubleshooting)

A total of up to 5 different interacting systems can be established and measured during a demo. Please prioritize your samples. In order to have a basis for your decision, we recommend measuring at least one sample whose affinity has been already characterized by other methods. Other orthogonal biophysical methods like ITC or SPR are preferred over pull-down or ELISA.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Experiment No** | **Principle Investigator or Group Name** | **Name and e-mail**  **of experimenter** | **Interaction characterized (yes, no)**  **If yes: method** | **Expected**  ***Kd*** | **Sample type**  **Concentration**  **Volume**  **Mol. Weight** | **Please add:**   * **To be labeled by NanoTemper** * **Already labeled (dye)** | **your purpose**  **(i.e. assay development, assay optimization, troubleshooting)** |
| **Example**  **0-Fluor.** | **Prof. XY** | **Dr. Scien Tist**  **Tist@university.edu** |  |  | **Protein (p38)**  **20 µM, 100 µl, 38 kDa** | **To be labeled by NanoTemper** | **e.g.**  **optimization required due to aggregation** |
| **Example**  **0-Titrant** |  |  | **Yes, ITC** | **120 nM** | **Compound (BIRB), 10 mM in DMSO, 50 µl 500 Da** |  |
| 1 –Fluor. |  |  |  |  |  |  |  |
| 1 –Titrant |  |  |  |  |  |  |
| 2 –Fluor. |  |  |  |  |  |  |  |
| 2 –Titrant |  |  |  |  |  |  |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Experiment No** | **Principle Investigator or Group Name** | **Name and e-mail**  **of experimenter** | **Interaction characterized (yes, no)**  **If yes: method** | **Expected**  ***Kd*** | **Sample type**  **Concentration**  **Volume**  **Mol. Weight** | **Please add:**   * **To be labeled by NanoTemper** * **Already labeled (dye)** | **your purpose**  **(i.e. assay development, assay optimization, troubleshooting)** |
| 3 –Fluor. |  |  |  |  |  |  |  |
| 3 –Titrant |  |  |  |  |  |  |  |
| 4 –Fluor. |  |  |  |  |  |  |  |
| 4 –Titrant |  |  |  |  |  |  |  |
| 5 –Fluor. |  |  |  |  |  |  |  |
| 5 –Titrant |  |  |  |  |  |  |  |

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