**Sample requirements for MST experiments**

Contact Information:

|  |  |
| --- | --- |
| Name |  |
| e-mail |  |
| Phone |  |
| Company/University |  |
| Department |  |
| Head of department |  |
| Street |  |
| Post code |  |
| City |  |
| Country |  |

Please summarize your interactions and the expected affinities in this table:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Target molecule | Interaction partner | Expected Kd | Method previously used to measure affinity |
| 1. |  |  |  |  |
| 2. |  |  |  |  |

For sample preparation please consider that all assays are set up in such a way that one molecule is fluorescent (or fluorescently labeled) and a non-fluorescent molecule is titrated. The titration usually begins at least 20 x concentration above the expected dissociation constant (Kd). NanoTemper Technologies provides labeling kits to label proteins with a MW of >10 kDa within 1 hour.

The fluorescent interaction partner is used at a concentration below the Kd. Minimum volume is 200 μl. Minimum amount of protein to be labeled: 100 μl with a concentration of 5-20 μM.

The non-fluorescent interaction partner is used at a concentration of at least 20 x above the Kd. Minimum volume is 30 μl. Please also prepare 50 ml of your reaction buffer. If you want to keep your molecules of interest confidential, name them X, Y, Z etc.

Sample description:

For most straightforward and effective assay optimization a well-characterized target molecule is beneficial. Therefore, we recommend answering as much questions as possible:

|  |  |
| --- | --- |
| Target molecule | |
| Name (full name and/or abbreviation) |  |
| Molecule class (e.g. kinase) |  |
| Origin (human, mouse, etc.) |  |
| Source (commercial (company and cat. no. or “in-house”) |  |
| If “in-house”: purification strategy |  |
| Purity information available? |  |
| Already fluorescent? How (e.g. GFP)? |  |
| Fusion protein with a tag? (histidine, GST, etc.)  *(important for selection of labeling strategy)* |  |
| Sequence available? If yes, please provide sequence information. *(important for selection of labeling strategy)* |  |
| Are lysines or cysteines located in the active site? *(important for selection of labeling strategy)* |  |
| Biochemical details | |
| Molecular weight [Da] |  |
| Molar extinction coefficient [M-1] |  |
| Multimer | Yes  No |
| Details (SS bridges, etc.), cysteines important for stability? |  |
| Requires reducing agent for stability? | Yes No |
| Co-factors required for binding/activity (e.g. Ca2+-dependent) |  |
| Availability and stability | |
| Sample dissolved in |  |
| Concentration [M] |  |
| Stability and storage (temperature, pH, salt, etc.) |  |
| Stable in pH 8.0? | Yes  No |
| Interaction partner | |
| Name (full name and/or abbreviation) |  |
| Class of interaction partners (e.g. small molecule) |  |
| Source (commercial (company and cat. no. or “in-house”) |  |
| Concentration [M] |  |
| Storage conditions |  |
| Kd (method and experimental conditions used for Kd determination) |  |
| Comments | |
|  | |

Please attach more than one form, if more than one target molecule or interaction partner will be sent (contact information need to be filled out only ones).

Contact

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