**Protocol for Fluorescence Anisotropy Measurements**

Use a 96-well plate to dilute the protein from a concentrated stock solution and then transfer to 384-well plate. Do not use the 96-well plate for the binding reaction. See table below for setting up the binding reaction; step 1 is to prepare a serial dilution of the protein ligand to be titrated:

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **96-well plate** | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |  |
| mutbzip-S310C Y307A | **A** | 0.0072 | 0.0286 | 0.1144 | 0.4578 | 1.8311 | 7.3242 | 29.297 | 117.188 | 468.75 | 1875 | 7500 | 30000 | nM |
| **B** | 0.0143 | 0.0572 | 0.2289 | 0.9155 | 3.6621 | 14.6484 | 58.594 | 234.375 | 937.5 | 3750 | 15000 | 60000 |  |
| mutbzip-S310C R314A | **C** | ***24*** | ***22*** | ***20*** | ***18*** | ***16*** | ***14*** | ***12*** | ***10*** | ***8*** | ***6*** | ***4*** | ***2*** |  |
| **D** | ***23*** | ***21*** | ***19*** | ***17*** | ***15*** | ***13*** | ***11*** | ***9*** | ***7*** | ***5*** | ***3*** | ***1*** |  |
| mutbzip-S310C Q321A | **E** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **F** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| mutbzip-S310C | **G** | 0.0014 | 0.0057 | 0.0229 | 0.0916 | 0.3662 | 1.4648 | 5.86 | 23.4375 | 93.75 | 375 | 1500 | 6000 |  |
| **H** | 0.0029 | 0.0114 | 0.0458 | 0.1831 | 0.7324 | 2.9297 | 11.72 | 46.875 | 187.5 | 750 | 3000 | 12000 |  |

1. Do serial dilution using two rows of a 96-well plate for each protein (CREB bZip S310C for binary titration, His6GST-CRTC55 for ternary titration). The order of dilution (red text) is D12 to C12 to D11 to C11... (see corresponding numbers cells A1:A12 and B1:B12).
2. Make DNA solution for binary titration or bZip-DNA for ternary titration. The concentration should be 6-fold concentrated than final concentration in the reaction.
3. Add 5 μl of DNA or bZip-DNA mixture into each well of 384-well plate.
4. Transfer 25 μl of protein from 96-well plate to the 384-well plate using multi-channel pipette. For example, wells of row A in 96-well plate (colored in black) go to A (odd numbered wells) in 384-well plate. Wells of row B in 96-well plate (colored in blue) go to A (even numbered wells).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **384-well plate (not all wells in plate shown below)** | | | | | | | | | | | | | | | | |
| nM |  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| mutbzip-S310C Y307A | **A** | 0.0060 | 0.0119 | 0.0238 | 0.0477 | 0.0954 | 0.1907 | 0.381 | 0.763 | 1.526 | 3.052 | 6.104 | 12.21 | 24.41 | 48.83 | 97.7 |
| **B** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| mutbzip-S310C R314A | **C** | 0.0060 | 0.0119 | 0.0238 | 0.0477 | 0.0954 | 0.1907 | 0.381 | 0.763 | 1.526 | 3.052 | 6.104 | 12.21 | 24.41 | 48.83 | 97.7 |
| **D** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| mutbzip-S310C Q321A | **E** | 0.0060 | 0.0119 | 0.0238 | 0.0477 | 0.0954 | 0.1907 | 0.381 | 0.763 | 1.526 | 3.052 | 6.104 | 12.21 | 24.41 | 48.83 | 97.7 |
| **F** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| mutbzip-S310C | **G** | 0.0012 | 0.0024 | 0.0048 | 0.0095 | 0.0191 | 0.0381 | 0.076 | 0.153 | 0.305 | 0.610 | 1.221 | 2.441 | 4.883 | 9.766 | 19.5 |
| **H** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

1. Read the plate after setting the appropriate measurement parameters for excitation and emission wavelengths, signal averaging, number of replicates, etc.