

# Studies of Chaperone-Cochaperone Interactions using Homogenous Bead-Based Assay

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## Materials

| Name                                   | Company                  | Catalog Number                 | Comments                              |
|--|--------------------------|--------------------------------|---------------------------------------|
| 384-well plates                        | Perkin Elmer             | 6008350                        | Assay volume 25 ml                    |
| Amicon 10.000 MWCO centrifugation unit | Millipore                | UFC901008                      | Concentrate protein                   |
| Ampicillin                             | Sigma                    | A0166                          | Antibiotics                           |
| Bacteria shaker Unimax 1010            | Heidolph                 |                                | Culture bacteria                      |
| cDNA clones for human FKBP51           | Source BioScience        | clone id: 5723416              | pCMV-SPORT6 vector                    |
| cDNA clones for human FKBP52           | Source BioScience        | clone id: 7474554              | pCMV-SPORT6 vector                    |
| Chemically Competent E. coli           | Invitrogen               | C602003                        | One Shot BL21 Star (DE3)              |
| Data analysis software                 | GraphPad Prism           | 9.0.0                          | Analysis data and make figures        |
| Data analysis software                 | Excel                    |                                | Analysis data                         |
| DMSO                                   | Supelco                  | 1.02952.1000                   | Dilute compounds                      |
| DPBS                                   | Gibco                    | 14190-144                      | Prepare solution                      |
| EDTA                                   | Calbiochem               | 344504                         | Prevent proteolysis during sonication |
| Glutathione                            | Sigma                    | G-4251                         | Elute GST-tagged proteins             |
| Glutathione donor beads                | Perkin Elmer             | 6765300                        | Donor bead                            |
| GST-trap column                        | Cytiva (GE Healthcare)   | 17528201                       | Purify GST-tagged proteins            |
| Isopropyl- $\beta$ -D-thiogalactoside  | Thermo Fisher Scientific | R0392                          | Induce protein expression             |
| LB Broth (Miller)                      | Sigma                    | L3522                          | Microbial growth medium               |
| PCR instrument                         | BIO-RAD                  | S1000 Thermal Cycler           | Amplification/PCR                     |
| PD-10 column                           | Cytiva (GE Healthcare)   | 17085101                       | Solution exchange                     |
| pGEX-6P-1 vector                       | Cytiva (GE Healthcare)   | 28954648                       | Plasmid                               |
| pGEX-6P-2 vector                       | Cytiva (GE Healthcare)   | 28954650                       | Plasmid                               |
| Plate reader                           | Perkin Elmer             | EnSpire 2300 Multilabel Reader | Read alpha plate                      |
| Plate reader software                  | Perkin Elmer             | EnSpire Manager                | Plate reader software                 |
| Plate reader software protocol         | Perkin Elmer             | Alpha 384-well Low volume      | Use this protocol to read plate       |
| PMSF                                   | Sigma                    | P7626                          | Prevent proteolysis during sonication |
| protease inhibitor cocktail            | Sigma                    | S8830                          | Prevent proteolysis during sonication |

|  |                 |             |   |
|--|-----------------|-------------|---|
| Sodium azide   | Sigma           | S2002       | As a preservative                       |
| Sodium cyanoborohydride (NaBH <sub>3</sub> CN)   | Sigma           | 156159      | Activates matrix for coupling           |
| Ten amino acid peptide NH <sub>2</sub> -EDASRMEEVD-COOH corresponding to amino acids 714-724 of human Hsp90 beta isoform | Peptide 2.0 inc |             | Synthesize Hsp90 C-terminal peptide     |
| Test-Tube Rotator  | LABINCO         |             | Make end-over-end agitation             |
| Tris-HCl   | Sigma           | 10708976001 | Block unreacted sites of acceptor beads |
| Tween-20   | Sigma           | P1379       | Prevent beads aggregation               |
| Ultra centrifuge Avanti J-20 XP  | Beckman Coulter |             | Centrifuge to get bacteria cell pellets |
| Ultrasonic cell disruptor  | Microson        |             | Sonicate cells to release protein       |
| Unconjugated acceptor beads  | Perkin Elmer    | 6762003     | Acceptor beads                          |
| XCell SureLock Mini-Cell and XCell II Blot Module  | Invitrogen      | EI0002      | SDS-PAGE                                |