**Why “Pre-spotted” Plates?**

Calibr has worked with dozens of screening partners to provide the ReFRAME collection for screening and it is intended to fuel hundreds of assays. Because the collection is finite, we provide the collection in nanomole quantities to prevent premature depletion of the library. The typical screening partner receives ~0.5 nanomoles of each compound during primary screening (i.e., **50 nL @ 10 mM**) and up to 4 nanomoles for “hits” during the campaign (includes primary screen, dose response experiments and counter screens). To achieve this, Calibr uses acoustic dispensing to “pre-spot” assay plates or intermediate plates for our partners. In addition to conserving the library, this format limits our partners’ need for automation and information management—all tracking, dosing and compound management are performed at Calibr. After the campaign, we work with our partners to provide larger quantities for the validated hits. However, until then the library is provided in two formats:

**Format 1,** **Pre-spotted Assay plates**: Calibr “dry spots” compound directly onto assay plates and ships to partner for assay. Optimal workflow when the assay can accommodate addition of compound at the start of the assay.

**Format 2,** **Pre-spotted Intermediate plates**:Calibr “dry spots” compound onto intermediate plates and ships to partner. The partner then resuspends compounds in assay buffer for transfer to the assay plate at the appropriate time. This workflow is necessary when the partner needs to delay addition of the compound to the assay well (e.g. some adherent cell-based assays).

Exceptions can be made. However, exceptions are rare and only made when the assay is considered very high priority with no other options. We typically will not accommodate assay well volumes greater than 50 μL and we expect our partners to conserve compound to the best of their ability. To date, a large majority of ReFRAME screens have been in 384- or 1536-well format.

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| **Summary of Standard Workflow** | |
| **1** | MTA Approval and partner completes *Assay Registration Description* Form |
| **2** | Partner completes *Plate Layout Form* and finalizes all details (i.e. plate type, volumes, controls, etc.). Typical concentration of compound transferred goes up to 10uM. Typical volume of compound transferred ranges from 2.5nL to 100nL from a 10mM source. |
| **3** | Partner sends Calibr plate and controls. Controls are given at 10mM in DMSO. |
| **4** | Calibr sends partner pre-spotted plates and compound keys (as blinded RFM IDs) for primary screen. Plates will be in singlicate in the format defined in *Plate Layout Form.* |
| **5** | Partner performs screen and select hits (up to 1% of the library) |
| **6** | Partner sends hitlist as RFM IDs |
| **7** | Calibr sends partner two sets of pre-spotted dose response plates. Sets are provided as replicate plates. Plates are prepared as 8-point 1:3 serial dilutions, starting at 10 μM unless otherwise discussed. |
| **8** | Collaborator sends Calibr dose response data via *Dose Response Data* form |
| **9** | Calibr sends collaborator structure and identities of confirmed hits |
| **10** | Final review of Assay Registration Description and publication date |
| **11** | In some cases, additional material for secondary assays can be provided by Calibr. Requests for additional material or other resources will be reviewed by the ReFRAME committee. Request are considered after steps 1 through 10 are complete. |