**Calibr ReFrame Assay Form**

A screening library of 12,000 molecules assembled by combining three databases (Clarivate Integrity, GVK Excelra GoStar and Citeline Pharmaprojects) to facilitate drug repurposing. \*\*Information provided in this form will be posted on the public reframe portal website. Example of a completed form is at the end of page. Please visit <https://reframedb.org/#/assays> to see more examples.

Screening Workflow: After MTA is signed, collaborator will provide plates and control. Primary screen of compounds will be pre-spotted and sent on assay ready plates. Collaborator will determine hits up to 1%. Hits will be pre-spotted in dose response in two sets. Structures and identities of the hits from dose response will be revealed. Any deviations from typical workflow require approval from the ReFrame Committee.

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| **General Information** | |
| **Assay ID** | **Provided by Calibr** |
| **Contact Name** | Click or tap here to enter text. |
| **Contact Phone** | Click or tap here to enter text. |
| **Contact Email(s)** | Click or tap here to enter text. |
| **Mailing Address** | Click or tap here to enter text. |
| **Assay Title** | Click or tap here to enter text. |
| **Assay Title Short** (less than 50 characters) | Click or tap here to enter text. |
| **Author** In format “Name, Titles (Location)” | Click or tap here to enter text. |
| **Summary** Brief sentence or two about the type of screen and the target.If secondary assay start with “Secondary assay for <assay title short>” | *Click or tap here to enter text.* |

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| **Compound Transfer Description** |

**Assay Volume (uL of volume at time of compound exposure)** (example: 50 μL)

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| *Ex: 50uL* |

**Primary Screening Volume Transfer from 10mM Source Plates.** (For cell-based assays, consider toxicity. A higher concentration (i.e. 10 μM) can lead to false negatives in many assays.)(example: 50 nL for 50 μL assay volume at 10 μM)

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| *Ex: 50nL for 50uL assay volume at 10uM.* |

**Plate Type. Format: Vendor Catalog number, (description)** (example: 384-well Corning 8793 (black solid bottom))

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| *Ex: 384-well Corning 8793 (black solid bottom)* |

**Dose Response Volume (High point and dilution). Dose response delivered as two singlicate dose sets.** (example: start at 10 μM (final concentration), 1:3 dilution, 8pt.)

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| *Ex: Start at 10uM (final), 1:3 dilution, 8pt.* |

By checking this box, I understand the primary screen of compounds will be pre-spotted and sent on assay ready plates. I, the collaborator, will determine hits up to 1%. Then, hits will be pre-spotted in dose response as two sets in singlicate.

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| **Assay Description** |

**Description (\*\*Word count between 100-200\*\*. The background biology needed to understand the screen. Should be detailed and specific to the assay. Keep general discussion of a disease to a minimum. Examples: the details and significance of the particular larval stage being studied in the assay; the mechanism of action of the enzyme being targeted by a biochemical screen; the benefits of your screen over other formats, details on cell type and modification if any; how your assay works to detect viability, activity or other factors etc.)**

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| Click or tap here to enter text. |

**Protocol/Workflow (A light-weight materials and methods section, but be as detailed as possible in sentence form. Please no bullet form.)**

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| Click or tap here to enter text. |

**Read out and Normalization (Clarify exactly what is being measured and how. Include information on normalization routines)**

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| Click or tap here to enter text. |

**Indication (Indicate disease that is targeting)**

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| Click or tap here to enter text. |

**Assay Type (Type of assay: cell based, biochemical, phenotypic…)**

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| Click or tap here to enter text. |

**AC50 Units (If another metric is used, please specify)**

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| Click or tap here to enter text. |

**Detection Method (Describe the way the assay is read)**

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| Click or tap here to enter text. |

**Detection Reagent (Provide the reagents used for detection)**

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| Click or tap here to enter text. |

**Components (Any species that are involved in the assay)**

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| Click or tap here to enter text. |

**Neutral Control (Position on plate, description, and amount transferred in nL)**

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| Click or tap here to enter text. |

**Stimulator or Inhibitor Control (Position on plate, description, source concentration provided by collaborator, final concentration in uM, and volume transferred in nL)**

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| Click or tap here to enter text. |

**DMSO Sensitivity (Highest percentage of DMSO that assay is tolerable at)**

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| Click or tap here to enter text. |

**Pre-screening Notes**

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| *Previous experiments showed a 10 fold decrease with positive control. Assay underwent several round of optimization to achieve a Z' score of 0.52. After pilot screen and consideration of control, hits will be selected when compounds reduce signal 7 standard deviations below mean. A 0.7% hit-rate was observed in the pilot with a 68% confirmation rate.)* |

**Assay Development Statistics (Z’, fold change, CV’s of strongest control)**

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| *Pilot (triplicate plates) had Z’ > 0.5 (10 fold change)* |

**Calibr Notes (leave blank)**

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| Click or tap here to enter text. |

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| **Pre-spotted plate description** |

**Description of pre-potted plates for single assay and corresponding counter screens. If this description is for a secondary or counter screen, please indicate the primary screen and leave the rest blank.**

**For primary screen 50 nL compound from 10 mM source in singlicate (layout: described in 1\_AssayRegistration.PlateLayout.xlsx)**

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| **ReFRAME Process**  **Summary of 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 20 uM 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. |

Example Calibr ReFrame Assay Form

A screening library of 12,000 molecules assembled by combining three databases (Clarivate Integrity, GVK Excelra GoStar and Citeline Pharmaprojects) to facilitate drug repurposing. \*\*Information provided in this form will be posted on the public reframe portal website. Example of a filled-out form is on the end of page.

|  |  |
| --- | --- |
| **General Information** | |
| **Assay ID** | **Provided by Calibr** |
| **Contact Name** | Dr. Jane Doe, PhD |
| **Contact Phone** | (123-456-7890) |
| **Contact Email(s)** | JaneDoe@scripps.edu |
| **Mailing Address** | 11119 N Torrey Pines Rd, La Jolla, CA 92037 |
| **Assay Title** | Crypto-C. hominis high-content imaging proliferation |
| **Assay Title Short** (less than 50 characters) | Crypto-C. hominis proliferation |
| **Author In format “Name, Titles (Location)”** | Jane Doe, PhD (California Institute for Biomedical Research (Calibr), La Jolla, CA) |
| **Summary Brief sentence or two about the type of screen and the target. If secondary assay start with “Secondary assay for <assay title short>”** | Secondary assay for Crypto-C. hominis proliferation. Phenotypic high-content imaging-based high-throughput screen against the protozoan pathogen C. parvum to find new small molecule inhibitors of Cryptosporidium, which causes diarrheal disease in humans. |

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| **Compound Transfer Description** |

**Assay Volume (uL of volume at time of compound exposure)**

|  |
| --- |
| *50uL* |

**Primary Screening Volume Transfer from 10mM Source Plates.**

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| --- |
| *50nL for 50uL assay volume at 10uM* |

**Plate Type. Format: Vendor Catalog number, (description)**

|  |
| --- |
| *384-well Corning 8793 (black solid bottom)* |

**Dose Response Volume (High point and dilution). Dose response delivered as two singlicate dose sets.**

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| --- |
| *Start at 10uM (final), 1:3 dilution, 8pt.* |

By checking this box, I understand the primary screen of compounds will be pre-spotted and sent on assay ready plates. I, the collaborator will determine hits up to 1%. Then, hits will be pre-spotted in dose response in duplicate.

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| --- |
| **Assay Description** |

**Description (\*\*Word count between 100-200\*\*. The background biology needed to understand the screen. Should be detailed and specific to the assay. Keep general discussion of a disease to a minimum. Examples: the details and significance of the particular larval stage being studied in the assay; the mechanism of action of the enzyme being targeted by a biochemical screen; the benefits of your screen over other formats, details on cell type and modification if any; how your assay works to detect viability, activity or other factors etc.)**

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| This is a secondary assay for hits active against C. parvum IOWA (Sterling and Bunch Grass Farm strains). Cryptosporidium parvum and the related species Cryptosporidium hominis cause diarrheal disease in humans. The current standard of care is ineffective in patients most at risk of cryptosporidiosis. This is a confirmatory secondary high-content imaging-based high-throughput screen of the hits from the full deck against the protozoan pathogen Cryptosporidium parvum. The assay measures inhibition of parasite proliferation within the human ileocecal colorectal adenocarcinoma cell line HCT-8 (a commonly used cell line for studying Cryptosporidium spp. in vitro. HCI detection of this cell-based assay allows for concomitant measurement of Cryptosporidium parasite inhibition and host cell cytotoxicity. |

**Protocol/Workflow (A light-weight materials and methods section, but be as detailed as possible)**

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| This specific ID is for C. hominis (TU502), which has been routinely passaged through neonatal gnotobiotic piglets at Tufts University by the Tzipori lab. For the assay, HCT-8 cells are seeded into 1536-well clear-bottom plates and incubated at 37C. 24 hours later, compounds are transferred (1.88 uM for primary screening and 25 uM top concentration for dose-response), and then excysted Cryptosporidium sporozoites and oocysts are dispensed onto cells. 48 hours later, cells are fixed with 4% paraformaldehyde. Cells are then washed, permeabilized with 0.25% Triton X-100 in PBS, blocked with SuperBlock (Thermo), and finally stained with a 1:10 dilution of Superblock with 1ug/mL FITC-tagged Vicia Villosa lectin and 3uM DAPI nuclear stain. Cells are then washed with PBS-T to remove residual stain and plates are sealed with aluminum adhesive seals. Plates are imaged (10x objective) on the CX5 Cellomics imager using the SpotDetector algorithm. Compounds that inhibit Cryptosporidium proliferation by >= 70% and do not inhibit HCT-8 growth by more than 40% are picked for dose response testing. |

**Read out and Normalization (Clarify exactly what is being measured and how. Include information on normalization routines)**

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| High-content imaging; normalized to neutral control minus inhibitor. |

**Indication (Indicate disease that is targeting)**

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| --- |
| Cryptosporidiosis |

**Assay Type (Type of assay: cell based, biochemical, phenotypic…)**

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| --- |
| cell based, High-Content Imaging |

**AC50 Units (If another metric is used, please specify)**

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| --- |
| AC50 in Molar |

**Detection Method (Describe the way the assay is read)**

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| High-Content Imaging; Nitazoxanide and floxuridine are used as positive controls, and DMSO is the neutral control. Spot Counts are normalized by Neutral Controls Minus Inhibitors. |

**Detection Reagent (Provide the reagents used for detection)**

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| FITC 485/521 |

**Components (Any species that are involved in the assay)**

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| C. hominis (TU502); HCT-8 cells; Detection reagent |

**Neutral Control (Position on plate, description, and amount transferred in nL)**

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| --- |
| 100nL of DMSO in column 22 |

**Stimulator or Inhibitor Control (Position on plate, description, source concentration provided by collaborator, final concentration in uM, and volume transferred in nL)**

|  |
| --- |
| 100nL of DMSO (worms added by collaborator) in column 23 |

**DMSO Sensitivity (Highest percentage of DMSO that assay is tolerable at)**

|  |
| --- |
| 1% |

**Pre-screening Notes**

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| --- |
| Previous experiments showed a 10 fold decrease with positive control. Assay underwent several round of optimization to achieve a Z' score of 0.52. After pilot screen and consideration of control, hits will be selected when compounds reduce signal 7 standard deviations below mean. A 0.7% hit-rate was observed in the pilot with a 68% confirmation rate. |

**Assay Development Statistics (Z’, fold change, CV’s of strongest control)**

|  |
| --- |
| Pilot (triplicate plates) had Z’ > 0.5 (10 fold change) |