# Human Fibroblast Growth Factor 4/β-Klotho for Endocrine FGF Signaling Reporter Assay System

# (FGFR4/β-Klotho)

**384-well Format Assays**
Product # IB43202

▪

**Technical Manual**
*(version 8.0i)*


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Nuclear Receptor & *In Vitro* Toxicology Solutions™

# Human FGFR4/β-Klotho for Endocrine FGF Signaling Reporter Assay System 384-well Format Assays

## *I. Description*

**▪ Background ▪**

The family of Fibroblast Growth Factors (FGFs) comprise approximately 23 members that are related by core sequence and structure conservation, with the majority of FGFs being secreted signaling proteins. Secreted FGFs are predominantly autocrine and paracrine factors, with only three members evolved to function as endocrine factors[1]. FGFs bind and activate FGF Receptors (FGFRs) which, themselves, are members of the family of high-affinity tyrosine kinase receptors[1].

Paracrine FGFs show high affinity towards the extracellular matrix (ECM) component heparin sulfate (HS) and are thus retained in the ECM and function locally. In contrast, the atypical endocrine subfamily of FGFs, that comprise FGF-19, FGF-21, and FGF-23, have reduced affinity for HS and can therefore escape from the ECM into the circulation to reach target distant organs[2]. However, this subfamily typically requires association with members of the Klotho family of proteins as cofactors for efficient binding to their cognate receptor(s)[1, 2, 3].

Aberrant FGF-19 signaling mediated by its receptor, FGFR4 and co-receptor β-Klotho, is known to be a driver of hepatocellular carcinoma (HCC)[4]. Consequently, FGF-19 and FGFR4/β-Klotho command considerable interest as therapeutic targets in drug development and drug safety screening.

**▪ The Assay System ▪**

This assay utilizes proprietary human cells that have been engineered to provide constitutive expression of the **Human Fibroblast Growth Factor Receptor 4 and β-Klotho**, referred to herein as FGFR4/β-Klotho. FGFR4 and β-Klotho are both single-pass transmembrane proteins. FGFR4 has an extracellular ligand-binding domain, transmembrane domain, and intracellular tyrosine kinase domain[1]. It has been established that FGFR4 association with the co-receptor β-Klotho generates a scaffold that is essential for **endocrine growth factor** binding interactions, such as those with FGF-19 and FGF-21[2, 3]. Following growth factor binding, the activated tyrosine kinase activities of the FGFR initiate intracellular signaling cascades that may include RAS-MAPK, PI3-AKT, PLCγ and/or STAT pathways[1]. For example, activation of the PLCγ pathway leads to an increase of intracellular calcium[1]. One prominent outcome of the FGF/FGFR > PLCγ pathway is that calcineurin, a calcium-dependent phosphatase, dephosphorylates and activates the transcription factor NFAT[5]. It is FGFR4 signal transduction *via* the $Ca^{+2}$·calcineurin / NFAT cascade that is exploited by the reporter cells provided in this kit.

INDIGO's FGFR4/β-Klotho for Endocrine FGF Signaling Reporter Cells contain the luciferase reporter gene functionally linked to an NFAT-AP-1 responsive promoter. Activated NFAT binds to the response elements to initiate the formation of a complete transcription complex that drives Luc gene expression. Quantifying relative changes in luciferase activity in the treated reporter cells relative to the untreated cells provides a sensitive, dose-dependent surrogate measure of drug-induced changes in FGFR4/β-Klotho activity. Accordingly, the principal application of this reporter assay is in the screening of test materials to quantify any functional interactions, either activating or inhibitory, that they may exert against FGFR4/β-Klotho or the coupled NFAT pathway.

INDIGO's Reporter Cells are transiently transfected and prepared as frozen stocks using a proprietary **CryoMite™** process. This cryo-preservation method allows for the immediate dispensing of healthy, division-competent reporter cells into assay plates. There is no need for intermediate treatment steps such as spin-and-rinse of cells, viability determinations or cell titer adjustments prior to assay setup.

This assay kit provides the convenience of an all-inclusive cell-based reporter assay system for FGFR4/β-Klotho endocrine FGF signaling. In addition to Reporter Cells, included are an optimized culture medium for use in reviving the cryopreserved cells, a culture medium for use in preparing test sample treatments, the physiological endocrine activator FGF-19, Luciferase Detection Reagents, and a cell culture-ready assay plate. To re-iterate, this assay is primarily focused on the *endocrine* signaling activities of FGFR/FGFs

## ▪ The Assay Chemistry ▪

INDIGO's receptor assays capitalize on the low background, high-sensitivity, and broad linear dynamic range of bio-luminescence reporter gene technology.

Reporter Cells incorporate the cDNA encoding beetle luciferase, a 62 kD protein originating from the North American firefly (*Photinus pyralis*). Luciferase catalyzes the mono-oxidation of D-luciferin in a $Mg^{+2}$-dependent reaction that consumes $O_2$ and ATP as co-substrates to yield oxyluciferin, AMP, $PP_i$, $CO_2$, and photon emission. Luminescence intensity of the reaction is quantified using a luminometer and is reported in terms of Relative Light Units (RLU's).

This assay kit features a luciferase detection reagent specially formulated to provide stable light emission between 5 and 90+ minutes after initiating the luciferase reaction. Incorporating a 5-minute reaction-rest period ensures that light emission profiles attain maximal stability, thereby allowing assay plates to be processed in batch. By doing so, the signal output from all sample wells, from one plate to the next, may be directly compared within an experimental set.

## ▪ Considerations for the Preparation and Automated Dispensing of Test compounds ▪

**Small-molecule** test compounds are typically solvated in DMSO at high concentrations; ideally 1,000x-concentrated stocks relative to the highest desired treatment concentration in the assay. Using high-concentration stocks minimizes DMSO carry-over into the assay plates.

Stocks of test materials that are **Protein** or **Poly-peptide** ligands, or **Antibodies**, should be solvated in aqueous buffered solutions with carrier protein (*e.g.,* PBS + 0.1% BSA).

For **384-well format assays** the user will choose to dilute master stocks using one of two alternative methods. The selection of dispensing method to be used will be dictated by the type of instrument that will be used. This Technical Manual provides detailed protocols for each of these two alternative methods:

*a.)* Assay setups in which a conventional **tip-based** instrument is used to dispense **µL volumes** of for both **small-molecule** and **proteinaceous** test samples into assay wells (protocol is presented in black text). Use **Compound Screening Medium (CSM)** to generate a series of **2x-concentration** test compound treatment media, as described in *Step 2a* of the **Assay Protocol**. The final concentration of DMSO carried over into assay reactions should not exceed 0.4%; strive to use 1,000x-concentrated stocks when they are prepared in DMSO.

*NOTE:* CSM is formulated to help stabilize hydrophobic test compounds in the aqueous environment of the assay mixture. Nonetheless, high concentrations of extremely hydrophobic test compounds diluted in CSM may lack long-term stability and/or solubility, especially if further stored at low temperatures. Hence, it is recommended that test compound dilutions are prepared in CSM immediately prior to assay setup and are considered to be 'single-use' reagents.

*and,*

***b.)*** **Acoustic transfer** or **Pin-based dispensing of nL volumes** of test compounds into assay wells (protocol is presented in blue text). Use CSM for proteinaceous test samples, or DMSO for small molecule test samples, to make a series of **1,000x-concentrated** test compound stocks that correspond to each desired final assay concentration, as described in *Step 2b* of the **Assay Protocol**.

### ▪ Considerations for Automated Dispensing of Other Assay Reagents ▪

When dispensing into a small number of assay plates, first carefully consider the dead volume requirement of your tip-based dispensing instrument before committing assay reagents to its setup. In essence, "dead volume" is the volume of reagent that is dedicated to the instrument; it will *not* be available for final dispensing into assay wells. The following Table provides information on reagent volume requirements, and available excesses on a *per kit* basis. Always pool the individual reporter cell suspensions and all other respective assay kit reagents before processing multiple 384-well assay plates.

| **Stock Reagent & Volume provided** | **Volume to be Dispensed** *(384-well plate)* | **Excess reagent available for instrument dead vol.** |
|---|---|---|
| *when using tip dispensing of test cmpds* **Reporter Cell Suspension** 7.5 ml | 15 µl / well 5.8 ml / plate | ~ 1.7 ml |
| *when using acoustic dispensing of test cmpds* **Reporter Cell Suspension** 15 ml | 30 µl / well 11.5 ml / plate | ~ 3.4 ml |
| **Detection Substrate** 7.8 ml | 15 µl / well 5.8 ml / plate | ~ 2 ml |

### ▪ Assay Scheme ▪

The *Day 1* preparation, volumes, and chronology of dispensed cells and test compounds are different between assay setups using a *tip-based dispenser* (**1a**) and those using an *acoustic transfer device* (**1b**). Following 22 -24 hours incubation Detection Substrate is added. Light emission from each assay well is quantified using a plate-reading luminometer.

**Figure 1a.** Assay workflow if using conventional **tip-based** dispensing of test compounds.

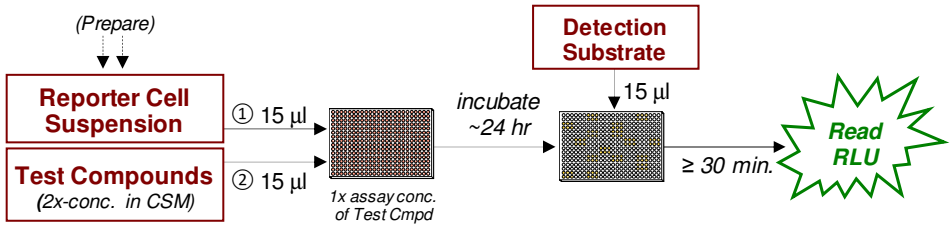
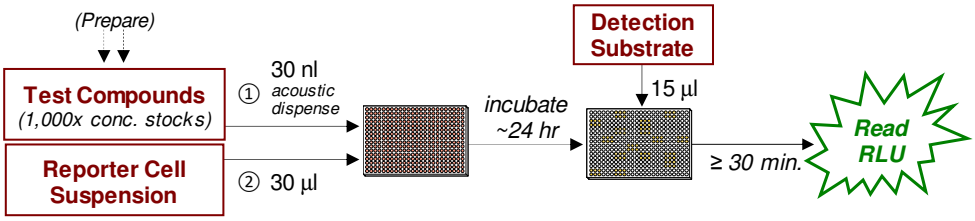



**Figure 1b.** Assay workflow if using **acoustic** dispensing of test compounds.



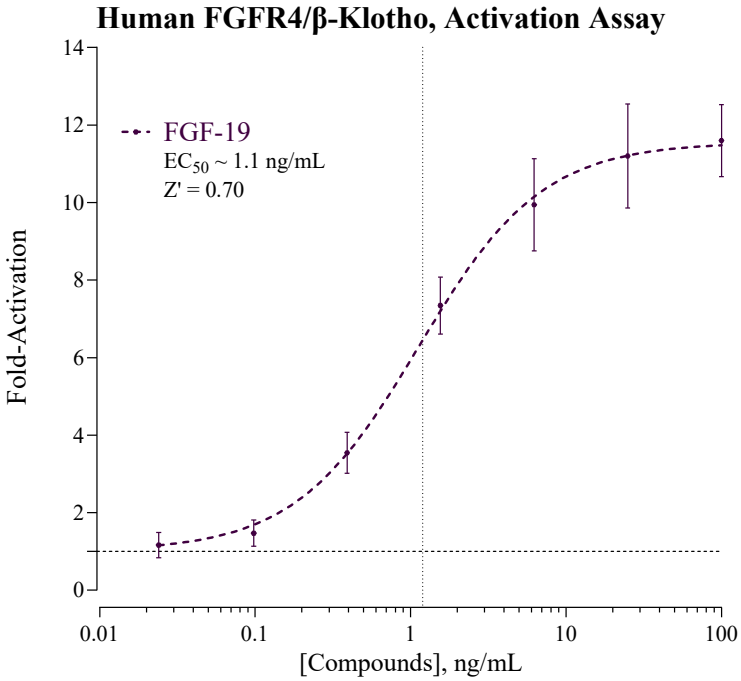



**Figure 2. Activation of FGFR4/β-Klotho.** Activation assay was performed according to the protocol provided in this Technical Manual using the reference activator FGF-19 (provided; Peprotech).

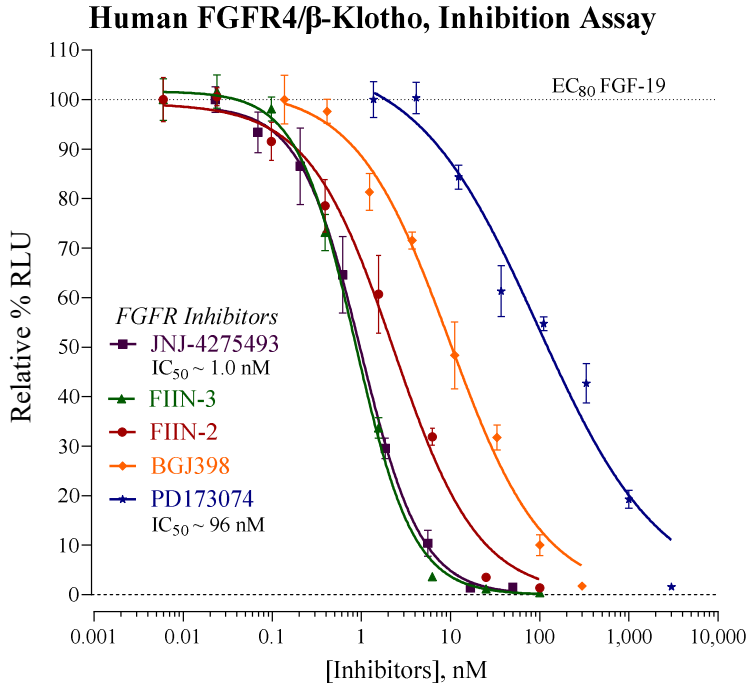



**Figure 3. Inhibition of FGFR4/β-Klotho.** FGFR4/β-Klotho cells were treated with an EC80 concentration of the reference activator FGF-19 and varying concentrations of the FGFR inhibitors JNJ-4275493, FIIN-3, FIIN-2, BGJ398 and PD173074 (all compounds obtained from Cayman Chemical). For both Activation and Inhibition assays, luminescence was quantified and values of average (n=3) relative light units (RLU), corresponding standard deviation (SD), Fold-Activation, Relative % RLU and Z'[5] values were calculated. GraphPad Prism software was used to plot data using the least-squares method of non-linear regression for Fold-Activation or Relative % RLU *vs.* $Log_{10}$ [Cmpd], and to determine $EC_{50}$ / $IC_{50}$ values.

## *II. Product Components & Storage Conditions*

This FGFR4/β-Klotho for Endocrine FGF signaling Assay kit contains materials to perform assays in a single 384-well assay plate.

***Cryopreserved mammalian cells are temperature sensitive!*** **To ensure maximal viability the tube of Reporter Cells must be maintained at -80°C until immediately prior to the rapid-thaw procedure described in *Step 2* of this protocol.**

Assay kits are shipped on dry ice. Upon receipt of the kit transfer it to -80°C storage. If you wish to first inspect and inventory the individual kit components, please be sure to first transfer and submerge the tube of reporter cells in dry ice.

The aliquot of Reporter Cells is provided as a single-use reagent. Once thawed, reporter cells can NOT be refrozen, nor can they be maintained in extended culture with any hope of retaining downstream assay performance. Therefore, extra volumes of these reagents should be discarded after assay setup.

The date of product expiration is printed on the Product Qualification Insert (PQI) enclosed with each kit.

| *Kit Components* | *Amount* | *Storage Temp.* |
|---|---|---|
| ▪ FGFR4/β-Klotho Reporter Cells | 1 x 1.0 mL | **-80°C** |
| ▪ Cell Recovery Medium (CRM) | 1 x 7 mL | -20°C |
| ▪ Compound Screening Medium (CSM) | 1 x 45 mL | -20°C |
| ▪ FGF-19, 100µg/ml (in PBS+0.1%BSA) (physiological activator of FGFR4/β-Klotho) | 1 x 60 µL | -20°C |
| ▪ Detection Substrate (Note: contains DTT) | 1 x 7.8 mL | **-80°C** |
| ▪ 384-well assay plate (white, sterile, cell-culture ready) | 1 | ambient |

## *III. Materials to be Supplied by the User*

The following materials must be provided by the user, and should be made ready prior to initiating the assay procedure:

***DAY 1***

▪ container of dry ice (see *Step 2*)

▪ cell culture-rated laminar flow hood.

▪ 37°C, humidified 5% $CO_2$ incubator for mammalian cell culture.

▪ 37°C water bath.

▪ 70% alcohol wipes

▪ Instrumentation suitable for dispensing 15 µl volumes

▪ disposable media basins, sterile.

▪ sterile multi-channel media basins *or* deep-well plates, *or* appropriate similar vessel for generating dilution series of reference compound(s) and test compound(s).

▪ *Optional:* inhibitor reference compound (*e.g.,* Figure 3)

▪ *Optional:* clear 384-well assay plate, cell culture treated, for viewing cells on *Day 2*.

***DAY 2*** plate-reading luminometer.

## *IV. Assay Protocol*

Review the entire Assay Protocol before starting. Completing the assay requires an overnight incubation. *Steps 1-8* are performed on ***Day 1***, requiring less than 2 hours to complete. *Steps 9-13* are performed on ***Day 2*** and require less than 1 hour to complete.

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**▪ A word about Inhibition-mode assay setups ▪**

Receptor inhibition assays expose the Reporter Cells to a fixed, sub-maximal concentration (typically between $EC_{50}$ – $EC_{85}$) of a known activator AND varying concentrations of the test compound(s) to be evaluated for chemical inhibition. This FGFR4/β-Klotho for Endocrine FGF Assay kit includes a 100µg/mL stock solution of FGF-19, a physiological activator of FGFR4/β-Klotho, that may be used to set up inhibition-mode assays. ~5 ng/mL FGF-19 typically approximates $EC_{80}$ in this assay and, therefore, is a suitable *final assay concentration* of activator to be used when screening test compounds for inhibitory activity.

Adding the challenge activator FGF-19 to the bulk suspension of Reporter Cells (*i.e.,* prior to dispensing into assay wells) is the most efficient and precise method of setting up inhibition assays, and it is the method presented in *Step 5b* of the protocol when performing tip-based dispensing, and *Step 6b* of the protocol when using an acoustic transfer device to dispense test compounds.

Note that when using a *tip-based instrument* for the dispensing of 2x-concentrated test compounds the cell suspension must also be supplemented with a **2x-**concentration (~ 10 ng / mL) of the challenge activator FGF-19.

When using an *acoustic transfer* device for the dispensing of 1,000x-concentrated test compounds the cell suspension should be supplemented with a **1x-**concentration (~ 5 ng/mL) of the challenge activator FGF-19.

**DAY 1 Assay Protocol:** All steps should be performed using aseptic technique.

**1.)** Remove **Cell Recovery Medium (CRM)** and **Compound Screening Medium (CSM)** from freezer storage and thaw in a 37°C water bath.

**2.) Prepare dilutions of test compounds:** Prepare Test Compound treatment media for *Activator-* or *Inhibition-mode* screens. NOTE that test and reference compounds will be prepared differently when using tip-dispensing *vs.* acoustic dispensing. Regardless of the method, the total DMSO carried over into assay reactions should not exceed 0.4%.

*a.* *Tip dispensing method*: In *Step 6*, 15 µl / well of the prepared treatment media is added to the assay that has been pre-dispensed with 15 µl /well of Reporter Cells. Hence, to achieve the desired *final* assay concentrations one must prepare treatment media with a **2x-**concentration of the test and reference material(s). Use **CSM** to prepare the appropriate dilution series. Plan dilution volumes carefully; this assay kit provides 45 ml of CSM.

*b.* *Acoustic dispensing method*: In *Step 6*, 30 nl / well of **1,000x**-concentrated test compound solutions are added to the assay plate using an acoustic transfer device.

**NOTE:* Stocks of test samples that are small-molecule chemicals / drugs are typically prepared in DMSO and, for acoustic transfer dispensing, we recommend that DMSO (not CSM) is used as the diluent to generate the desired series of 1,000x-treatment concentrations. However, stocks of test samples that are solvated in aqueous solution, such as protein ligands and antibodies, should be further diluted using CSM (*not* DMSO).

**Preparing the positive control:** This assay kit includes a 1,000x concentrated stock of the polypeptide FGF-19, 100 µg/mL prepared in PBS+0.1%BSA. The following 7-point treatment series, with concentrations generated using serial 3-fold dilutions, provides a complete dose-response: 100, 25.0, 6.25, 1.56, 0.391, 0.098, and 0.024 ng/mL. Always include 'no treatment' (or 'vehicle') controls.

**APPENDIX 1a** provides an example for generating such a dilution series to be used when *tip-dispensing* compound solutions prepared in CSM (15 µl / well).

*(continued …)*

**APPENDIX 1b** provides an example for generating such a series of 1,000x-concentrated solutions of compounds to be used when performing *acoustic dispensing* (30 nl / well). As noted in *Step 2b*, use CSM to dilute sample and reference stocks that have been prepared in aqueous solutions (*e.g.*, protein ligands, antibodies, *etc.*), or use DMSO to further dilute sample stocks that were initially solvated in DMSO (*e.g.* small molecule chemicals).

**When using *tip-based* instrumentation for dispensing test compounds …**

**3.)** *First*, retrieve the tube of **CRM** from the 37°C water bath, sanitize the outside with a 70% ethanol swab.

*Second*, retrieve **Reporter Cells** from -80°C storage and immerse in dry ice to transport the tube to a laminar flow hood. Perform a *rapid thaw* of the frozen cells by transferring a **6.5 ml** volume of 37°C CRM into the tube of frozen cells. Recap the tube of Reporter Cells and place it in a 37°C water bath for 5 - 10 minutes. The resulting volume of cell suspension will be 7.5 ml.

**4.)** Retrieve the tube of Reporter Cell Suspension from the water bath. Sanitize the outside surface of the tube with a 70% alcohol swab, then transfer it into the cell culture hood.

**5.)** Gently invert the tube of cells several times to gain a homogenous suspension.

***a.*** **for *Activation*-mode assays**: Dispense **15 µl / well** of cell suspension into the assay plate.

*~ or ~*

***b.*** **for Inhibition-mode assays**: Supplement the bulk volume of Reporter Cells suspension with a 2x-concentration of the challenge activator (refer to "*A word about Inhibition-mode assay setup*", pg. 8). Dispense **15 µl / well** of cell suspension into the assay plate.

**6.)** Dispense **15 µl / well** of 2x-concentrated treatment media (from *Step 2a*) into the assay plate.

**When using an *acoustic transfer* device for dispensing test compounds …**

**3.)** Dispense **30 nl / well** of the 1,000x-concentrated compounds (from *Step 2b*) into the assay plate.

**4.)** *First*, retrieve the tube of **CRM** from the 37°C water bath, sanitize the outside with a 70% ethanol swab;

*Second*, retrieve **Reporter Cells** from -80°C storage and immerse in dry ice to transport the tube to a laminar flow hood. Perform a *rapid thaw* of the frozen cells by transferring a **6.5 ml** volume of 37°C CRM into the tube of frozen cells. Recap the tube of cells and place it in a 37°C water bath for 5 - 10 minutes.

**5.)** Retrieve the tube of cell suspension from the water bath. Sanitize the outside surface of the tube with a 70% alcohol swab. Add an additional **7.5 ml** of **CSM** to the tube. The resulting volume of cell suspension will be 15 ml.

**6.)** Gently invert the tube of cells several times to gain a homogenous cell suspension.

***a.*** **for *Agonist*-mode assays**: Dispense **30 µl / well** of cell suspension into the assay plate that has been pre-dispensed with test compounds.

*~ or ~*

***b.*** **for *Inhibition*-mode assays:** First supplement the bulk volume of FGFR4/β-Klotho Reporter Cells suspension with the challenge activator **FGF-19** to achieve an $EC_{50}$ – $EC_{80}$ concentration (refer to "*A word about inhibition-mode assay setups*", pg. 8). Then dispense **30 µl / well** of the supplemented cell suspension into the assay plate that has been pre-dispensed with test compounds.

*(continued …)*

*NOTE:* Take special care to prevent cells from settling during the dispensing period. Allowing cells to settle during the transfer process, and/or lack of precision in dispensing uniform volumes across the assay plate *will* cause well-to-well variation (= increased Standard Deviation) in the assay.

*NOTE:* Following the dispensing of Reporter Cells and test compounds INDIGO recommends performing a *low-speed* spin of the assay plate (with lid) for ≤ 1 minute using a room temperature centrifuge fitted with counter-balanced plate carriers.

**7.)** Transfer the assay plate into a 37°C, humidified, 5% $CO_2$ incubator for 22 - 24 hours.

*NOTE:* Ensure a high-humidity (≥ 70%) environment within the cell culture incubator. This is critical to prevent the onset of deleterious "edge-effects" in the assay plate.

**8.)** For greater convenience on *Day 2*, retrieve **Detection Substrate** from freezer storage and place in a dark refrigerator (4°C) to thaw overnight.

## DAY 2 Assay Protocol:

Subsequent manipulations do *not* require special regard for aseptic technique and may be performed on a bench top or in a **fume hood**.

**9.)** Approximately 30 minutes before intending to quantify receptor activity remove **Detection Substrate** from the refrigerator and place it in a low-light area so that it may equilibrate to room temperature.

*NOTE:* Do NOT actively warm Detection Substrate above room temperature. If this solution was not allowed to thaw overnight at 4°C, a room temperature water bath may be used to expedite thawing.

**10.)** Set the plate-reader to "luminescence" mode. Program the instrument to perform a single 5 second "plate shake" prior to reading the first assay well. Set the read time to 0.5 second (500 mSec) per well, *or less*.

**11.)** Following 22 - 24 hours of incubation dispense **15 µl / well** of **Detection Substrate** into all wells of the assay plate.

*NOTE:* 'Detection Substrate' contains a high concentration of DTT, which produces a strong odor that some users may find objectionable. It is advised to work in a **fume hood** when dispensing it into the assay plate and throughout the following 'plate rest' period.

*NOTE:* Scattered micro-bubbles in the assay wells will not pose a problem. However, bubbles covering the surface of the reaction mix, or large bubbles clinging to the side walls of the well, will cause lens-effects that will degrade the accuracy and precision of the assay data. It is advised to perform a final *low-speed* spin of the assay plate (with lid) for ≤ 1 minute using a room temperature centrifuge fitted with counter-balanced plate carriers.

**12.)** Allow the plate(s) to rest at room temperature for 30 minutes. Do not shake the assay plate(s) during this period.

*NOTE:* the luminescent signal is somewhat unstable during the first 30 minutes of the luciferase reaction, however, after the initial 30-minute reaction period the luminescence signal achieves a stable emission output.

**13.)** Quantify luminescence.

**14.)** Data analyses.

## *V. Related Products*

| *Product No.* | *Product Descriptions* |
|---|---|
| **Fibroblast Growth Factor 4/β-Klotho Assay for Endocrine FGF Signaling** | |
| IB43201 | FGFR4/β-Klotho Assay for Endocrine FGF signaling<br>1x 96-well format assay |
| IB43202 | FGFR4/β-Klotho Assay for Endocrine FGF signaling<br>1x 384-well format assays |
| **Fibroblast Growth Factor 4 Assay for Paracrine FGF Signaling** | |
| IB43001 | FGFR4 Assay for Paracrine FGF signaling<br>1x 96-well format assay |
| IB43002 | FGFR4 Assay for Paracrine FGF signaling<br>1x 384-well format assays |
| **Fibroblast Growth Factor 4/α-Klotho Assay for Endocrine FGF Signaling** | |
| IB43101 | FGFR4/α-Klotho Assay for Endocrine FGF signaling<br>1x 96-well format assay |
| IB43102 | FGFR4/α-Klotho Assay for Endocrine FGF signaling<br>1x 384-well format assays |
| **Fibroblast Growth Factor Receptor 1 / 2 Assay for Paracrine FGF Signaling** | |
| IB21001-32 | FGFR1/2 Assay for Paracrine FGF Signaling<br>3x 32 assays in 8-well strips (96-well plate format) |
| IB21001 | FGFR1/2 Assay for Paracrine FGF Signaling<br>1x 96-well format assay |
| IB21002 | FGFR1/2 Assay for Paracrine FGF Signaling<br>1x 384-well format assays |
| **Fibroblast Growth Factor 1c/β-Klotho Assay for Endocrine FGF Signaling** | |
| IB22001-32 | FGFR1c/β-Klotho Assay for Endocrine FGF signaling<br>3x 32 assays in 8-well strips (96-well plate format) |
| IB22001 | FGFR1c/β-Klotho Assay for Endocrine FGF signaling<br>1x 96-well format assay |
| IB22002 | FGFR1c/β-Klotho Assay for Endocrine FGF signaling<br>1x 384-well format assays |

*(continued)*

| Fibroblast Growth Factor 1c/α-Klotho Assay for Endocrine FGF Signaling | |
|---|---|
| IB40001 | FGFR1c/α-Klotho Assay for Endocrine FGF signaling<br>1x 96-well format assay |
| IB40002 | FGFR1c/α-Klotho Assay for Endocrine FGF signaling<br>1x 384-well format assays |
| Bulk volumes of FGFR Assay Reagents may be custom manufactured to accommodate any scale of HTS. ***NOTE:*** **Single receptor FGFR1 or FGFR2 Assays are available upon request**. Please Inquire. | |

| NFAT Assays<br>(recommended for receptor-specificity counter-screening) | |
|---|---|
| IB18001-32 | NFAT Reporter Assay System<br>3x 32 assays in 8-well strips (96-well plate format) |
| IB18001 | NFAT Reporter Assay System<br>1x 96-well format assay |

| LIVE Cell Multiplex (LCM) Assay | |
|---|---|
| LCM-01 | Reagent volumes sufficient to perform **96** Live Cell Assays in 1x96-well, or 2x48-well, or 3x32-well assay plate formats |
| LCM-05 | Reagent in **5x bulk volume** to perform **480** Live Cell Assays contained in 5 x 96-well assay plates |
| LCM-10 | Reagent in **10x bulk volume** to perform **960** Live Cell Assays contained in 10 x 96-well assay plates |

| INDIGlo Luciferase Detection Reagent | |
|---|---|
| LDR-10, -25, -50, -500 | INDIGlo Luciferase Detection Reagents, available in 10 mL, 25 mL, 50 mL, 500 mL, and larger custom volumes |

Please refer to INDIGO Biosciences website for updated product offerings.

**www.indigobiosciences.com**

## *VI. Limited Use Disclosures*

Products offered by INDIGO Biosciences, Inc. are for RESEARCH PURPOSES ONLY – not for therapeutic, diagnostic, or contact use in humans or animals.

"CryoMite" is a Trademark ™ of INDIGO Biosciences, Inc. (State College, PA, USA).

Product prices, availability, specifications, claims and technical protocols are subject to change without prior notice. The printed Technical Manual provided in the kit box will always be the most current version available.

## *VII. Citations*


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[2] Xie, Y, *et al.* (2020) FGF/FGFR signaling in health and disease. Signal Transduction and Targeted Therapy (Springer Nature) **5**:181, 1-38.

[3] Shi, *et al.* (2018) A systematic dissection of sequence elements determining β-Klotho and FGF interaction and signaling. Scientific Reports. **8**:11045.

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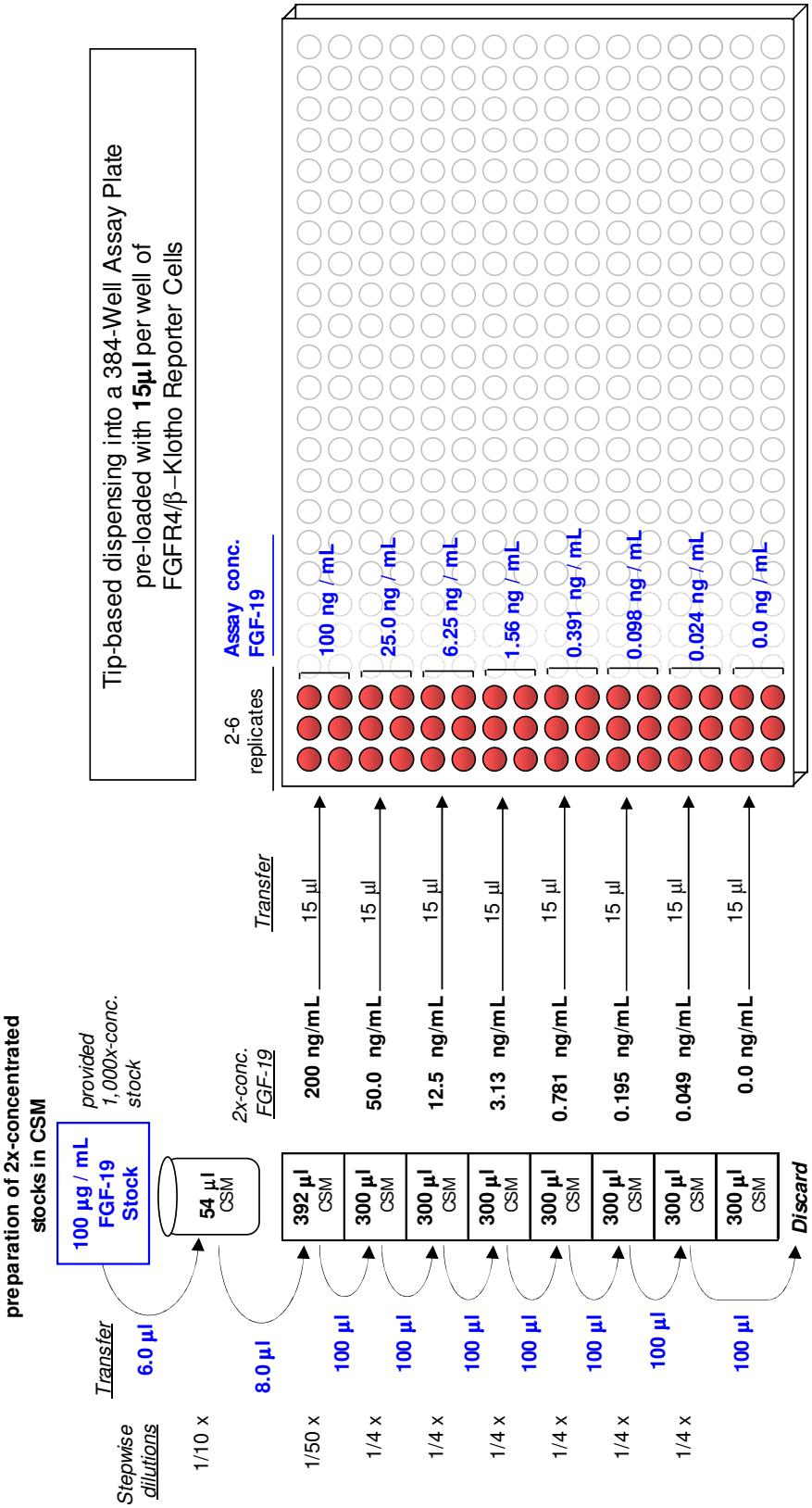
[5] Park JY, *et. al.* (2020) The Role of Calcium-Calcineurin-NFAT Signaling Pathway in Health and Autoimmune Disease. Frontiers in Immunology.:doi:10.3389/fimmu.2020.00195.

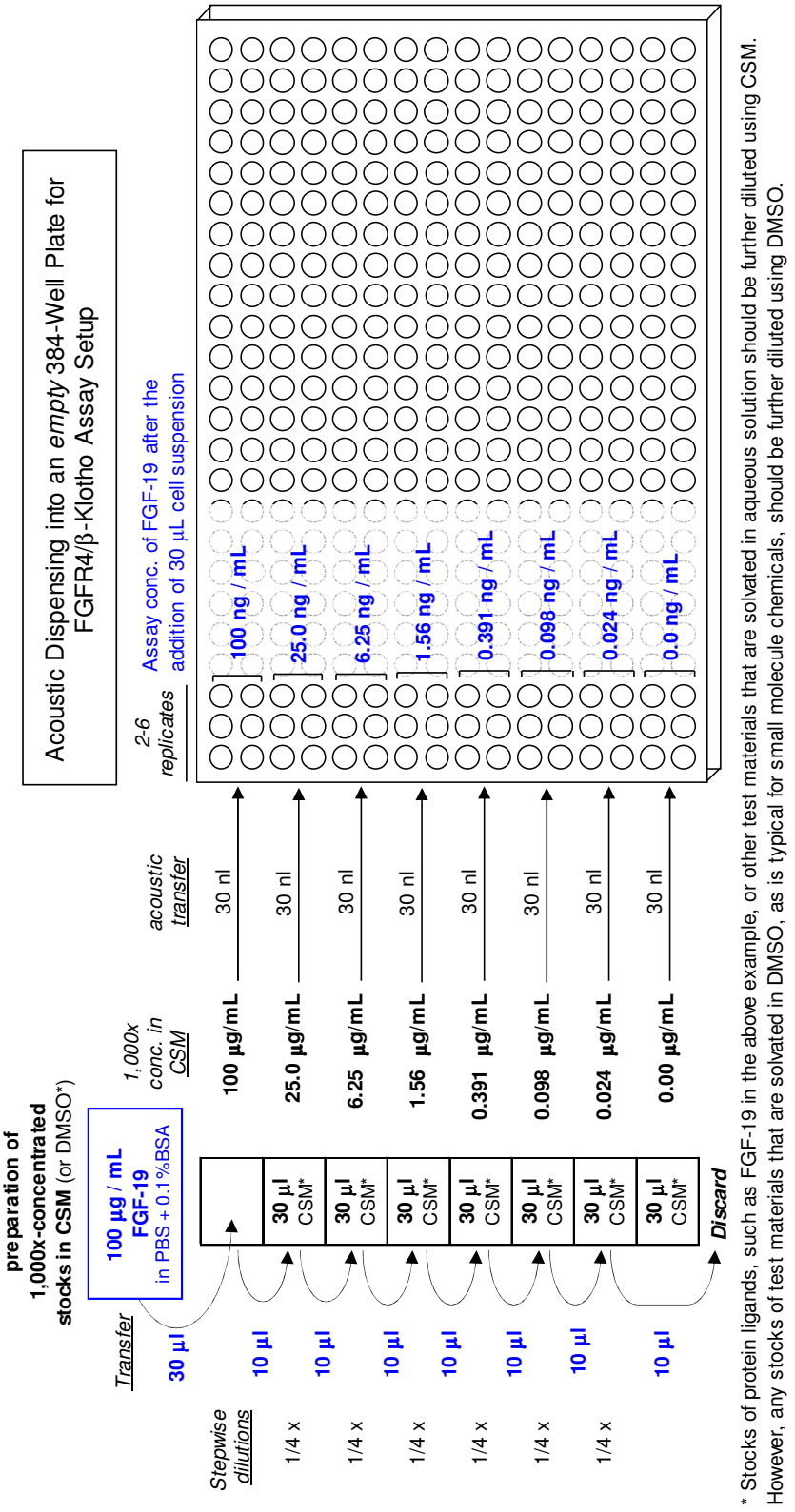
[6] Zhang JH, *et al.* (1999) A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. J Biomol Screen.:**4**(2), 67-73.

$$Z' = 1 - [3*(SD^{Reference} + SD^{Untreated}) / (RLU^{Reference} - RLU^{Untreated})]$$

**APPENDIX 1a for tip-based dispensing.** Example scheme for the serial dilution of the reference agonist FGF-19 into CSM to generate **2x-concentrated** treatment media. 15 µl / well are dispensed into assay plates using a *tip-based* instrument.



**APPENDIX 1b for acoustic dispensing.** Example scheme for the serial dilution of the reference agonist FGF-19 (a protein) into CSM to generate **1,000x-concentrated** stocks. 30 nl / well are pre-dispensed into assay plates using an acoustic transfer device. **NOTE:* Stocks of small-molecule test drugs are typically prepared in DMSO, and **DMSO** (*not* CSM) should be further used to generate the desired series of 1,000x-treatment concentrations.




* Stocks of protein ligands, such as FGF-19 in the above example, or other test materials that are solvated in aqueous solution should be further diluted using CSM. However, any stocks of test materials that are solvated in DMSO, as is typical for small molecule chemicals, should be further diluted using DMSO.