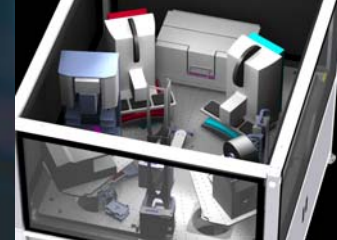


# A Total Solution to Provide High Content Primary and Secondary Screening of a Compound Library

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## Abstract

G-protein coupled receptors (GPCRs) represent the largest and most frequently screened class of receptors. TTP LabTech recently published a novel approach using the Acumen Explorer with Invitrogen's GeneBLAzer technology to measure GPCR activation in cell-based assays. This is multiplexed with a cytotoxicity readout to reduce false positives commonly identified in bulk fluorescence assays. TTP LabTech and Velocity11 have designed a fully automated, single platform using this assay to perform both primary and hit confirmation screens on a 400,000 compound library in only one week.

## Primary Screening and Hit Confirmation Procedure

Incubator 1: compound plates (240, 1536 format) at 18°C, dry atmosphere.

Incubator 2: assay plates (240, 384 format) at 37°C and 5% CO<sub>2</sub>.

Step 1: medium is exchanged for assay medium on plate washer.

Step 2: **VPrep**® adds 100nL of compounds into 50ul cell solution, with mixing.

Step 3: incubation at 37°C and 5% CO<sub>2</sub> for 4 h.

Step 4: **VPrep** adds 10µL of GeneBLAzer dye solution.

Step 5: incubation at room temperature, light protected, for 1 h.

Step 6 (optional): **PlateLoc**® seals plates

Step 7: **Acumen Explorer**™ analyses the assay plates

Step 8: **Hit ID** software prepares hit list

Step 9: **mosquito**® X1 cherry-picks compounds

Step 10: **mosquito**® HTS serially dilutes the compounds.

Procedure is repeated as outlined above.

## Key Benefits

- 400,000 compounds screened and confirmed in 1 week
- 100,000 compounds per day in primary screen
- 4,000 IC<sub>50</sub>s per day for hit confirmation
- 100-fold reduction of cell culture requirements
- Primary and secondary screens on a single platform
- Multiplexed high content readouts
- Assay-ready volumes eliminate dilution steps

## Automation Overview

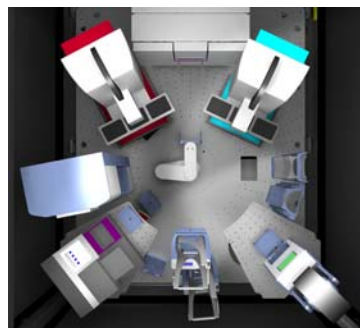
BioCel® 1800 and VWorks™:

➤ Flexibility and highest possible throughput on a very small footprint

• Combination of high speed robot and powerful software assures efficient and protective handling of valuable cell plates

• Liquid handling designed to guarantee cell viability and reliable results

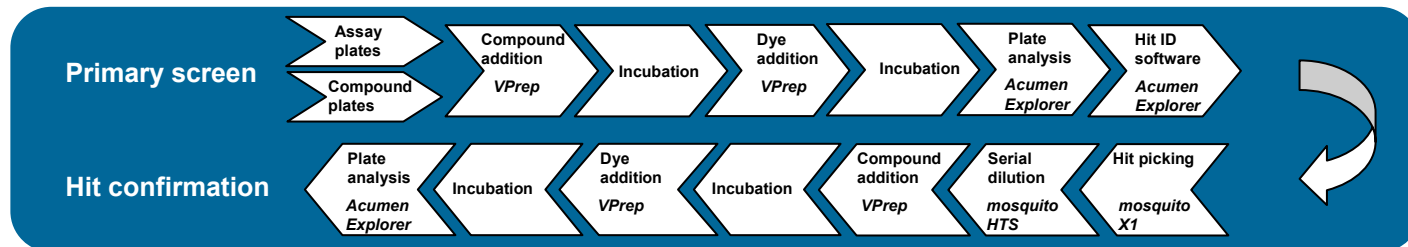
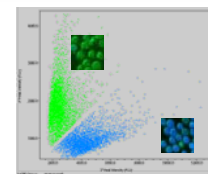
• Below-deck incubators allow for optimal storage conditions while keeping overall footprint small



## High Content Screening

TTP LabTech Acumen Explorer 405:

- Measures GPCR-activity and cytotoxicity simultaneously
- Uses partial well strip analysis for primary screen taking 4 minutes per 384-well assay plate
- Uses whole well analysis for hit confirmation screen to obtain Z' > 0.8 taking 10 minutes per plate
- Both screens require only 1,000 cells per well, using only 5 flasks of cells for a 100,000 compound primary screen



## Plate Replication, Hit Picking and Serial Dilution

Velocity11 VPrep:

- 384 fixed tip head pipettor
- Reformats 1536 compound plates to 384 assay plates
- Compounds (100nL) added direct to assay plates
- Throughput of up to 300 plates per day



TTP LabTech Mosquito X1:

- Single low-volume pipette tip for hit-picking
- Samples 100nL of hit compound from primary screen
- Transfers 32 compounds to an empty 384 well intermediary plate
- Approximately 7 seconds per hit transfer



TTP LabTech Mosquito HTS:

- Serially dilutes hits across plate using DMSO in a final volume of 800nL
- Transfers 100nL of each concentration to cells in assay plate
- Disposable tips ensure zero cross-contamination and no washing steps
- Serial dilution time about 6.5 minutes per plate



## Component list

- Velocity11 BioCel 1800
- Velocity11 VWorks
- Velocity11 VPrep
- Velocity11 PlateLoc
- TTP LabTech mosquito X1
- TTP LabTech mosquito HTS
- TTP LabTech Acumen Explorer
- Plate washer/bulk dispenser
- Below-deck incubators