# Method to Determine Liquid Transfer Volume with Pin Tools: FITC in DMSO

## I. OVERVIEW

### A. Purpose

This technote describes the protocol for determining the volume transferred by the pins of a V&P Pin Tool. The included information regarding the reagents, equipment, and methodology can also be used to validate the pins. This protocol is written for a 384 well pin tool but can be modified for 96 or 1536 pin tools.

### B. Protocol

1. A polypropylene source plate is prepared with dilutions of FITC in 100% DMSO.
2. Black polystyrene assay plates are filled with 75 ul 0.1 M Tris-HCl, pH 8.0 per well.
3. Pin tool is dipped 3X into source plate to wet the pins and pick up FITC.
4. The FITC is then transferred into assay plate by moving pin tool up and down 3X to mix FITC into the wells filled with Tris.
5. Pins are cleaned between transfers according to standard protocols outlined in Technote 67B.
6. Assay plates are covered and stored at room temperature in the dark until ready to read fluorescence.
7. Mix the well contents with an Orbital Shaker for microplates, minimum of 3 minutes (or let sit over night to allow for complete mixing.)
8. Read at 485/535 ex/em using Tris-HCL as a blank.

### C. Standard Curve

To determine the best dilutions of FITC to use for the control standard curve, an initial test should be run with two-fold serial dilutions of FITC (such as 24 two-fold serial dilutions). This will determine the best range, over the 24 dilutions, to use as the control standard curve.

## II. MATERIALS, REAGENTS AND EQUIPMENT

### A. Materials

- Greiner Bio-One 384-well polypropylene clear plate (Cat. No. 781076)
- Greiner Bio-One 384-well polystyrene black plate (Cat. No. 781076)
- 50 mL Conical Tubes (BCT-P50RS)
- 500 mL VWR Bottles (Cat. No. 89000-238)

**B. Reagents**

- Fluorescein 5-isothiocyanate, Isomer I "FITC" (Sigma F7250)
- Dimethyl Sulfoxide (Sigma D2650)
- Tris-HCl, 1 M Stock Solution, pH 8.0 (Sigma T3038)
- V&P Pin Cleaning Solution (VP 110)
- Isopropyl alcohol
- Distilled water (***not*** Deionized)

**C. Equipment**

- 12-Channel Pipette (Thermo Finn pipette Model 4510 Multi-Channel Digital Pipette)
- Plate filling instrument such as V&P Scientific's 384 Dispensing Manifold with Mounting Jig (VP 179BJD and VP 179A)
- 2 or 3 V&P Scientific Wash Reservoirs such as VP 549ETOH-4 and VP 549H2O-4
- 3 or 4 Blot Stations (VP 540DB)
- Robotic Workstation such as V&P's Pin Tool Robot (VP 903B)
- Microplate Orbital Shaker such as V&P's VP 325A (96), VP 325B (384), VP 325C (1536)
- Microplate Reader (such as PerkinElmer Victor 3)

## III. PROCEDURES

### A. Preparing Pin Tool

The pin tool should be washed according to standard cleaning protocols (Technote 67B), with the exception that the pin tool should be sonicated for 15 minutes and soaked in VP 110 cleaning solution for 30 minutes.

### B. Preparing FITC Dilutions

Dissolve the FITC (250 mg/ bottle) in 4 mL of DMSO, by pipetting the DMSO into the container. Leave the container in a dark place at room temperature for 2 hours (or until all the FITC has dissolved.) Pour the entire dilution into a 500 mL VWR bottle. Add enough DMSO to fill the bottle to the 500 mL mark. This will be the stock solution (0.5 mg/mL) for source plates. Store the FITC stock with aluminum foil and kept in a dark place to avoid photo-bleaching (ie. refrigerator).*

Use the following table as a guideline to prepare the FITC dilution according to the type of pin being tested:

| Concentration (mg/mL) | Pin Type |
|---|---|
| 0.01 | FP4S2000 |
| 0.01 | FP4S1000 |
| 0.5 | FP3S500 |
| 0.5 | FP3S200 |
| 0.5 | FP3S100 |
| 0.5 | FP4 |
| 0.5 | FP3 |
| 0.5 | FP1S50 |
| 0.5 | FP1S40 |
| 0.5 | FP1S30 |
| 0.5 | FP1S20 |
| 0.5 | FP1S10 |
| 0.5 | FP1S6 |
| 0.5 | FP1 |
| 0.5 | FP8 |
| 0.5 | FP9 |
| 0.005 | FP6 & FP Series |

The same concentrations can be used for the hydrophobic coated counterparts. If testing requires a different FITC concentration, serial dilutions can be done to prepare the appropriate concentration using the stock solution. For example, all E-clip FP and FP6 series require a FITC dilution of 0.005 mg/mL for the source plate. A table showing how to prepare the appropriate dilutions is on the following page.

| Use | Stock Conc. (mg/mL) | Stock Volume (mL) | Diluent (mL) | Final Volume (mL) | Final Conc. (mg/mL) |
|---|---|---|---|---|---|
| Source Plate FP1 - FP3S500 | 62.5 | 4 | 496.0 | 500 | 0.500 |
| Source Plate FP4S1000 & FP4S2000 | 0.5 | 1 | 49.0 | 50 | 0.010 |
| Source Plate E-Clip Pins | 0.5 | 0.5 | 49.5 | 50 | 0.005 |
| Standard Curve Plate | 62.5 | 4 | 496.0 | 500 | 0.500 |

## C. Preparing Source Plate

Use a Greiner 384 polypropylene plate for the source plate. Pipette 75 uL of the appropriate FITC dilution into each well using the 12-channel pipettor. Cover this plate and leave in the dark until starting assay. One source plate will suffice per assay.

## D. Preparing Assay Plates

To prepare the assay plates, fill each daughter plate with 75 uL of 0.1 M, pH 8.0, Tris-HCl buffer. 0.1 M Tris buffer can be made by diluting 100 mL of 1M Tris into 900 mL distilled water. Dispense the Tris-HCl into the plates using the VP 179BJD and VP 179A or another suitable liquid dispenser.

## E. Preparing Standard Curve

The standard curve should be prepared according to the table provided in the TechNote "267-1 V&P Pin Volume Data Template". This data template is available as an Microsoft Excel file that can be downloaded from V&P's website or emailed by a V&P customer service representative (see contact information in Technical Assistance on page 5). A standard curve is generated with two-fold serial dilutions of FITC in DMSO. The dilutions are prepared in 1.5 ml tubes. Then 10 ul volumes of each dilution are added to 65ul of Tris using a pipettor. The slope and y-intercept values from the linear range of the standard curve are used to determine the mean volume of FITC transferred by each pin tool. These values are calculated in the "384 Stnd Crv-1" sheet of the 267-1 Pin Volume Data Template.

## F. Preparing Liquid Handling Workstation

### Attaching and Aligning Pin Tool

Attach the pin tool via the appropriate VP mounting plate to the liquid handling workstation or "robot" to be used in assay. Use the VP 903R-384 to center the pin tool relative to a plate position on the deck. Make sure the pins are aligned in all dimensions (x, y, z, rotation, and tilt).

### Configuring Plates

Follow the instructions of VP Technotes 67B to setup the plates, blot stations, and washing reservoirs. These parts are configured such that the pin tool steps are:

1. Source Plate
2. Assay Plate
3. Blot
4. Wash
5. Blot
6. Wash
7. Blot
8. Wash
9. Blot

Blot stations are VP 540DB and wash stations are the VP 549ETOH-4 and VP 549H2O-4 reservoirs. The first VP 549H2O-4 wash station is a 1:1 mix of DMSO and distilled water. The second VP 549H2O-4 wash station is distilled water. The third VP 549ETOH-4 wash station is 99.9% isopropyl alcohol. Make sure that the succeeding wash station liquid level is slightly higher than the previous wash station liquid level. The liquid levels should be very close to the top, but should not touch the rim, and should account for displacement volume caused by the pins. Exact volumes are not important as long as succeeding liquid levels are higher than previous wash stations. And that the liquid level is high enough to wash the pins of where the FITC reached.

Alternatively, a "two wash reservoir" cleaning method can be used. Retain the first and third washes, eliminate the water wash. Set up as follows: blot, VP 549H2O-4 wash station with 1:1 mix of DMSO and distilled water, blot, VP 549ETOH-4 wash station is 99.9% isopropyl alcohol, and blot.

**Teach Plate Positions**

The pin tool must be mounted and centered in order to set up the plate positions. Plate positions may vary from assay to assay depending on the pin tool used or the type of plate.

Source and Assay Plates

- The lower height setting should be such that all pins touch the bottom of the plate. The upper setting should be such that all pins clear the liquid in the plate.
- The configuration for the source and assay plates are the same.
- Speed settings should be set to one speed for both plates, with last withdrawal from liquid being the most important.
- Deck cycles (pins in/out of liquid) should be 3 and delay set to a time appropriate to the viscosity of the solution.

Blot Stations

- The lower setting should be such that all pins touch the blot station and presses the paper down to the polypropylene pad. The upper setting should clear the pins of the station.
- Speed settings should be set to 100% for all entries.
- Deck cycles should be 1 with a delay of 3 seconds.

Wash Stations

- The lower settings should be such that the bottom plate is slightly above but not touching the reservoir. The upper setting should clear the pins of the reservoir.
- Speed settings should be set to 100% for all entries.
- Deck cycles should be 3 a delay of 1 second.

Max Z Height Setting

- Max Z height setting should be set so that the pins clear the highest object on the deck. This is generally the wash reservoir.

**Configure Routine**

Plate routine should be set for deck locations 1 through 9. Routines should be set to 3 cycles (for triplicates).

**G. Running Assay**

When all parameters are set up and tested, the pin tool assay can be run. When the pin tool robot is running, the assay plates must be changed after each replicate. Cover the recipient plates after each replicate until ready to be read.

**H. Reading Plates**

Read the plates in a fluorescent plate reader such as a PerkinElmer Victor 3 with the following settings in the protocol: Ex/Em 485/535, 0.1sec, 384 well plate. After the reading the plate, open up results file in the explorer, and export it, naming the file according to the plate label.

## IV. Analyzing Data

The 267-1 Pin Volume Data Template is setup so that pasting the raw plate data into the appropriate fields will perform the necessary calculations. Use "384 Stnd Crv-1" sheet for standard curve plate calculations and "Plate Calculations" sheet for test plates. Use the "Individual Pin CVs" sheet to determine %CV for each pin or an entire pin tool of pins. The "Volumetric Calculations" sheet will calculate the volume transferred by the test pins. This sheet will also highlight pins which are outside a defined validation range so that these can be replaced with "good" pins.

## TECHNICAL ASSISTANCE

If assistance is required, contact V&P Scientific, Inc. at 858-455-0643 or sales@vp-sci.com.