Setup Guide

Automation of Kinase Selectivity Profiling System with PIPETMAX®

Before you begin, please ensure that you are using the most current version of this document.

- Familiarize yourself with the operation of PIPETMAX® by reading the *PIPETMAX® 268 User's Guide*.
- Familiarize yourself with the Promega® Kinase Selectivity Profiling System and ADP-Glo™ Kinase Assay by reading the Kinase Selectivity Profiling System Technical Manual TM421.
- Follow all manufacturers' instructions for safe use of equipment, reagents, and materials.
- See **Appendix** for links to additional references and video tutorials.

Technical literature related to the Promega Kinase Selectivity Profiling System is available at **www.promega.com/protocols**

Technical literature related to PIPETMAX is available at **www.gilson.com**.

Please visit **www.gilson.com/kinaseprofilingbundle** to verify that you are using the most current version of this technical document.

E-mail Promega technical services (**techserv@promega.com**) or Gilson technical support (**techsupport@gilson.com**) if you have questions on use of this system.

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Description

The Kinase Selectivity Profiling System is used to generate kinase profiles for lead compounds. Two protocols have been created and validated by Promega® for use on PIPETMAX®. Each protocol will array compounds in duplicate against up to 16 kinases. The KSPS Single Dose Profile will prepare up to 20 compounds at a single concentration, while the KSPS Dose Response Profile will prepare up to 2 compounds at 10 concentrations. Both protocols also include two controls: +ATP/Substrate, kinase, -compound (maximum kinase activity control), and +ATP/Substrate, -kinase, -compound (background control)

Automation Protocol Overview

1. Prepare compound serial dilution (KSPS Dose Response Profile).
2. Transfer compound and control buffers to assay plate.
3. *(Prompt)*
   Centrifuge the assay plate. Remove tips from the tip waste bin. Load Kinase and Substrate strips onto Code 496PT rack.
4. Prepare Kinase and Substrate strips.
5. Transfer Kinase to assay plate.
6. *(Prompt)*
   Centrifuge the assay plate, mix for 2 min, and incubate for 10 min at room temp. Remove tips from the tip waste bin.
7. Transfer ATP/Substrate to assay plate.
8. *(Prompt)*
   Centrifuge the assay plate, mix 2 min, and incubate for 60 min at room temperature. Remove tips from the tip waste bin.
9. Transfer ADP-Glo Reagent to assay plate.
10. *(Prompt)*
    Centrifuge the assay plate, mix for 2 min, and incubate for 40 min at room temperature. Remove tips from the tip waste bin. Load Kinase Detection Reagent to Reagent Source Plate
11. Transfer Kinase Detection Reagent to assay plate.
12. *(Prompt)*
    END of automated protocol. Centrifuge the assay plate, mix for 2 min, and incubate for 30 min at room temp.

Equipment, Materials and Reagents

Required

Part	Part Number	Notes
PIPETMAX® 268	32100000 (standard cover), 32100001 (cover with cut-outs) or 32100002 (no cover with external safety interlock)	Required
MAX8x20 Pipette Head	FC10022	Required
MAX8x200 Pipette Head	FC10022	Required
Code 496PT Rack (Freezer Block)	32000238	Required
PIPETMAX 268 Tray 384-Well	32000091	Required
TRILUTION® micro	32000321(for tablet) or 32000320 (for PC)	Required
PIPETMAX Tip Adapter Block	F172211	Total of 4 Required
DSL10ST Tips	F172211	Required
DS200ST Tips	F172311	Required

Recommended

These parts are not required to run the Promega® Kinase Profiling System on PIPETMAX. Because of the number of tips that could be used in the protocol, note that the tip storage riser is strongly recommended.

Part	Part Number	Notes
Tip storage riser for PIPETMAX	32000177	Strongly Recommended
PIPETMAN pipettes	Various	Recommended

Promega Parts

Part	Part Number	Notes
Kinase Selectivity Profiling Systems	Visit Promega **here** for more information	Many options
Promega GloMax® Discover	Visit Promega **here** for more information	Recommended

Parts Sold Separately

Part	Part Number	Notes
Corning Low Volume Assay Plate	Corning 4512	Required
Corning 96-well V-Bottom Plate	Corning 3897	Required

Download Online

Part	Link	Notes
PIPETMAX Protocol(s)	http://www.gilson.com/kinaseprofilingbundle	Free of charge
Promega Excel Worksheets	Contact Promega for up-to-date information	Free of charge

Refer to Promega Technical Manual TM421 for complete instructions for Kinase Selectivity Profiling System

Kinase Profiling Protocols

KSPS Single Dose Profile

This procedure uses the **KSPS Single Dose Profile** protocol for PIPETMAX®. This protocol has been developed and validated by Promega® and is available for download at **www.gilson.com/kinaseprofilingbundle**. If you have not already done so, please import the protocol into TRILUTION® micro.

Overview

The Single Dose Profile protocol will array up to 20 compounds at a single concentration in duplicate against up to 16 kinases (2 strips). Two controls will also be generated for each kinase: (1) +ATP/Substrate, +kinase, -compound (maximum kinase activity), and (2) +ATP/Substrate, -kinase, -compound (background)). Compounds 1–10 are tested against the first kinase strip, while compounds 11–20 are tested against the second kinase strip. Optionally, compounds 1–10 can be used for both the first and second kinase strip, by setting the "Same Set of Inhibitors for Both Kinase Strips" variable to True.

Variables

Variables can be set each time the protocol is run, providing options for the number of compounds and kinase strips to evaluate.

Number of Kinase Test Strips: 1-2

Description: The number of kinase test strips used. Each kinase test strip has a matching substrate/cofactor strip. 1 kinase test strip can test 1–10 compounds, while 2 strips are needed for 11–20.

Number of Compounds: 1–20

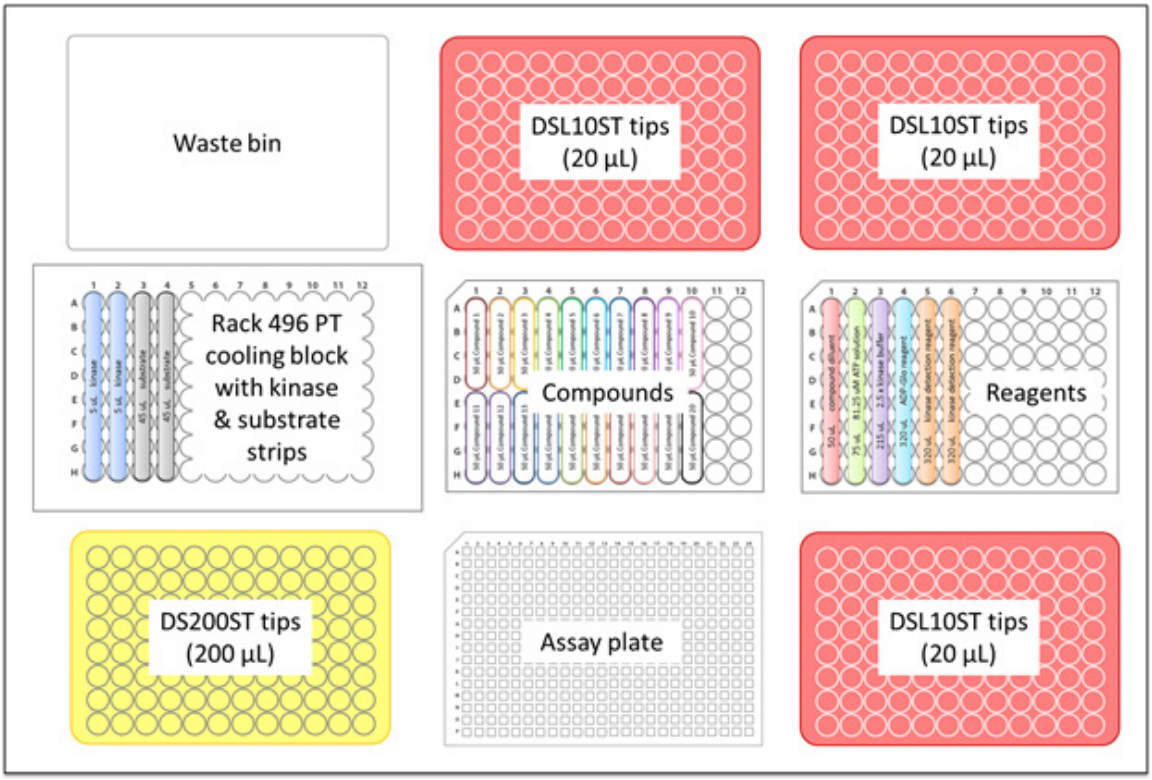
Description: The number of compounds to be tested. 1–10 compounds can be used with 1 kinase strip. 11–20 compounds require a second kinase test strip.

Same Set of Inhibitors for Both Kinase Strips: True/False

Description: When the Same Set of Inhibitors is being used for Both Kinase Strips (variable set as TRUE), the protocol is limited to 10 compounds.

Bed Layout

There are nine positions on the bed of PIPETMAX instrument. Each position can hold an item that conforms to the footprint dimensions defined in ANSI SLAS 1-2004 (R2012), such as a 96 well or 384 well microplate (appendix). In the Kinase Selectivity Profiling System (KSPS) single dose assay automated with PIPETMAX® all nine of the bed positions are occupied. Note that depending on how many compounds are being tested additional blister packs of tips may be required during the run.



1. Tip waste
2. DSL10ST tips in tip adapter block
3. DSL10ST tips in tip adapter block
4. Passive cooling block: Strip tubes containing kinase enzyme (columns 1 and 2) and substrate (columns 3 and 4) in Gilson Code 496PT rack (chilled to 0°C–4°C)
5. Inhibitors plate: Test compounds in positions A1:H10 of 96 well V-bottom plate, Corning 3897
6. Reagent source plate: Assay reagents in positions A1:H6 of 96 well V-bottom plate, Corning 3897
7. DS200ST tips in tip adapter block
8. Assay plate: 384 well assay plate, white, Corning 4512
9. DSL10ST tips in tip adapter block

Preparation

It is recommended to store the Code 496PT rack at -20°C. Place the Kinase and substrate strips on the Code 496PT rack and move to 4°C when assay preparation begins. Ensure that no clips or screws prevent the Code 496PT rack from seating properly onto the bed. Confirm that centrifuge for 384-well plates and the incubator (plate warmer) is set to 22°C–25°C. Check the 384-well plate before use—if it rocks when placed on the bench (i.e., it is warped) choose a different plate. Prepare the necessary reagents according to the volumes needed (below).

1. Prepare ***4x Kinase Buffer***

Component	*Volume*	*Final Concentration*
Nuclease-free water	495 µL	
5x Reaction Buffer A	2000 µL	4x
DTT, 0.1M	5 µL	200 µM
Total volume	**2.5 mL**	

2. Prepare ***2.5x Kinase Buffer***

Component	*Volume*	*Final Concentration*
Nuclease-free water	750 µL	
4x Kinase Buffer	1250 µL	2.5x
Total volume	2.0 mL	

   a. Dispense 215 µL aliquots of ***2.5x Kinase Buffer*** to column 3 (A3:H3) of Reagent source plate (96 Well V Bottom Plate; Corning 3897), position 6

3. Prepare 1x Kinase Buffer with 5% DMSO (a.k.a. ***compound diluent***)

Component	*Volume*	*Final Concentration*
Nuclease-free water	2100 µL	
4x Kinase Buffer	750 µL	1x
100% DMSO*	150 µL	5%
Total volume	**3.0 mL**	

*Notes: DMSO may be replaced with an alternate vehicle of choice.

   a. Dispense 300 µL aliquots of ***compound diluent*** to column 1 (A1:H1) of Reagent source plate (96 Well V Bottom Plate; Corning 3897), position 6

4. Prepare ***80 µM ATP***

Component	Volume	Final Concentration
Nuclease-free water	744 µL	
Ultra Pure ATP (10 mM)*	6 µL	1x
Total volume	**750 µL**	

*Notes: thaw 10 mM Ultra Pure ATP and keep on ice. (Aliquot if desired and) freeze remainder at -20°C.

a. Dispense 75 µL aliquots of ***80 uM ATP*** to column 2 (A2:H2) of Reagent source plate (96 Well V Bottom Plate; Corning 3897), position 6

5. Prepare ***compound(s)***.

Component	Volume	Final Concentration
Nuclease-free water	175 µL	
4x Kinase buffer	62.5 µL	1x
1 mM compound in 100% DMSO	12.5 µL	50 µM*
Total volume	**250 µL**	

*Notes: final concentration of DMSO is 5%. DMSO may be replaced with the vehicle of choice.

a. Dispense 50 µL aliquots of compound(s) to Inhibitors plate (96 Well V Bottom Plate; Corning 3897), bed position 5

Compound 1	A1-D1	**Compound 11**	E1-H1
Compound 2	A2-D2	**Compound 12**	E2-H2
Compound 3	A3-D3	**Compound 13**	E3-H3
Compound 4	A4-D4	**Compound 14**	E4-H4
Compound 5	A5-D5	**Compound 15**	E5-H5
Compound 6	A6-D6	**Compound 16**	E6-H6
Compound 7	A7-D7	**Compound 17**	E7-H7
Compound 8	A8-D8	**Compound 18**	E8-H8
Compound 9	A9-D9	**Compound 19**	E9-H9
Compound 10	A10-D10	**Compound 20**	E10-H10

6. Prepare ***ADP-Glo Reagent***
    a. Thaw and equilibrate to room temperature.
    b. Dispense 320 µL aliquots of ***ADP-Glo Reagent*** to column 4 (A4:H4) of Reagent source plate (96 Well V Bottom Plate; Corning 3897), position 6
7. Prepare ***Kinase Detection Reagent***
    a. As per **instructions**: thaw and equilibrate to room temperature. Light sensitive: prevent extended exposure to light.
    b. Add entire volume to (room temperature) amber bottle containing kinase detection substrate to form kinase detection reagent. Use immediately or dispense into 3 ml aliquots and store at -20°C.
    c. Dispense 320 µL aliquots of ***Kinase Detection Reagent*** to columns 5 and 6 (A5:H5) of Reagent source plate (96 Well V Bottom Plate; Corning 3897), position 6

Refer to Promega Technical Manual TM421 for complete instructions for Kinase Selectivity Profiling System

KSPS Dose Response Profile

This procedure uses the KSPS Dose Response Profile protocol for PIPETMAX. is protocol has been developed and validated by Promega and is available for download at **www.gilson.com/kinaseprofilingbundle**. If you have not already done so, please import the protocol into TRILUTION micro.

Overview

The Dose Response Profile protocol will prepare a serial dilution of up to 2 compounds at 10 concentrations according to the Serial Dilution Factor variables (below). The compounds will be arrayed in duplicate against up to 16 kinases (2 strips). Two controls will also be generated for each kinase: +ATP/Substrate, +kinase, -compound (maximum kinase activity), and +ATP/Substrate, -kinase, -compound (background)). If using one compound and 2 kinase strips, the serial dilution will be arrayed against both kinase strips. If using two compounds and two strips, compound 1 will be arrayed against kinase strip 1, and compound 2 will be arrayed against kinase strip 2.

Variables

Variables can be set each time the protocol is run, providing options for the number of compounds and kinase strips to evaluate.

Number of Kinase Test Strips: 1–2

Description: The number of kinase test strips used. Each kinase test strip has a matching substrate/cofactor strip.

Number of Compounds: 1–2

Description: The number of compounds to be tested.

Double the dilution rows if using one compound: True/False

Description: Doubling the rows of the compound in the serial dilution plate from 2 to 4 will decrease the time it takes to array the compound to the assay plate because it will be arrayed four tips at a time instead of two tips at a time.

Compound1 Serial Dilution Factor: 2–20

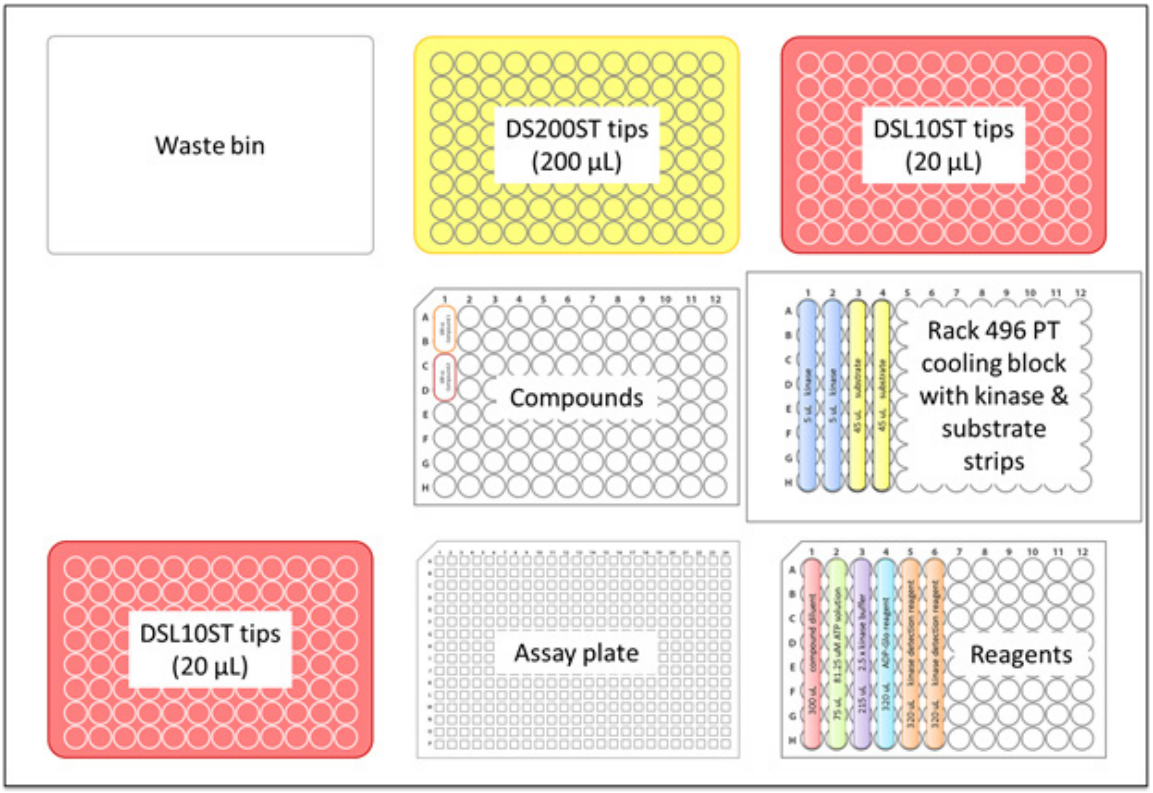
Description: Compound1 dilution factor (1:2 to 1:20 fold dilution)

Compound2 Serial Dilution Factor: 2–20

Description: Compound2 dilution factor (1:2 to 1:20 fold dilution)

Bed Layout

There are nine positions on the bed of PIPETMAX instrument. Each position can hold an item that conforms to the footprint dimensions defined in ANSI SLAS 1-2004 (R2012), such as a 96 well or 384 well microplate. In the Kinase Selectivity Profiling System (KSPS) dose-response assay automated with PIPETMAX® seven of the bed positions are occupied.



1. Tip waste bin
2. DS200ST tips in tip adapter block
3. DSL10ST tips in tip adapter block
4. Empty
5. Compound dilution plate: Test compounds in positions A1:D1 of 96 well V-bottom plate, Corning 3897.
6. Passive cooling block: Strip tubes containing kinase enzyme (columns 1 and 2) and substrate (columns 3 and 4) in Gilson Code 496PT rack (chilled to 0°C–4°C)
7. DSL10ST tips in tip adapter block
8. Assay plate: 384 well assay plate, white, Corning 4512
9. Reagent source plate: Assay reagents in positions A1:H6 of 96 well V-bottom plate, Corning 3897

Preparation

It is recommended to store the Code 496PT rack passive cooling block at -20°C. Place the kinase and substrate strips on the Code 496PT rack and move to 4°C when assay preparation begins. Ensure that no clips or screws prevent the Code 496PT rack from seating properly onto the bed. Confirm that centrifuge for 384-well plates and the incubator (plate warmer) is set to 22°C–25°C. Check the 384-well plate before use—if it is warped, choose a different plate. Prepare the necessary reagents according to the volumes needed (below).

1. Prepare ***4x Kinase Buffer***

Component	*Volume*	*Final Concentration*
Nuclease-free water	495 µL	
5x Reaction Buffer A	2000 µL	4x
DTT, 0.1M	5 µL	200 µM
Total volume	**2.0 mL**	

2. Prepare ***2.5x Kinase Buffer***

Component	*Volume*	*Final Concentration*
Nuclease-free water	750 µL	
4x Kinase Buffer	1250 µL	2.5x
Total volume	**2.0 mL**	

   a. Dispense 215 µL aliquots of ***2.5x Kinase Buffer*** to column 3 (A3:H3) of Reagent source plate (96 Well V Bottom Plate; Corning 3897), position 9.

3. Prepare 1x Kinase Buffer with 5% DMSO (a.k.a. ***compound diluent***).

Component	*Volume*	*Final Concentration*
Nuclease-free water	2100 µL	
4x Kinase Buffer	750 µL	1x
100% DMSO*	150 µL	5%
Total volume	**3.0 mL**	

*Notes: DMSO may be replaced with an alternate vehicle of choice.

   a. Dispense 300 µL aliquots of ***compound diluent*** to column 1 (A1:H1) of Reagent source plate (96 Well V Bottom Plate; Corning 3897), position 9.

4. Prepare ***80 µM ATP***

Component	*Volume*	*Final Concentration*
Nuclease-free water	744 µL	
Ultra Pure ATP (10 mM)	6 µL	1x
Total volume	**750 µL**	

Notes: thaw 10mM Ultra Pure ATP and keep on ice. (Aliquot if desired and) freeze remainder at -20°C.

   a. Dispense 75 µL aliquots of ***80 uM ATP*** to column 2 (A2:H2) of Reagent source plate (96 Well V Bottom Plate; Corning 3897), position 9.

5. Prepare ***compound***.

Component	*Volume*	*Final Concentration*
Nuclease-free water	175 µL	
4x Kinase buffer	62.5 µL	1x
1 mM compound in 100% DMSO	12.5 µL	50 µM*
Total volume	**250 µL**	

*Notes: final concentration of DMSO is 5%. DMSO may be replaced with the vehicle of choice.

   a. Dispense 100 µL aliquots of compound(s) to Compound dilution plate (96 Well V Bottom Plate; Corning 3897), position 5.

      1. Compound 1 into wells A1-B1 (for one compound) or A1 – D1 (for two compounds or the double the dilutions scenario).

      2. Compound 1: A1-B1 (or A1:D1 if the variable Double the dilution rows if using one compound is set to True).

      3. Compound 2: C1-D1.

6. Prepare ***ADP-Glo Reagent***

   a. Thaw and equilibrate to room temperature.

   b. Dispense 320 µL aliquots of ***ADP-Glo Reagent*** to column 4 (A4:H4) of Reagent source plate (96 Well V Bottom Plate; Corning 3897), position 9.

7. Prepare ***Kinase Detection Reagent***

   a. As per **instructions**: thaw and equilibrate to room temperature. Light sensitive: prevent extended exposure to light.

   b. Add entire volume to (room temperature) amber bottle containing kinase detection substrate to form kinase detection reagent. Use immediately or dispense into 3 ml aliquots and store at -20°C.

   c. Dispense 320 µL aliquots of ***Kinase Detection Reagent*** to columns 5 and 6 (A5:H5) of Reagent source plate (96- Well V Bottom Plate; Corning 3897), position 9.

Frequently Asked Questions (FAQ)

Q: Can I prepare additional 5X Reaction Buffer A?

A: Yes. **5X Reaction Buffer A**

200 mM Tris-HCl (pH 7.5)

100 mM MgCl2

0.5 mg/mL BSA

Q: The instructions specify particular labware for the compound plate, reagent plate and assay plate. Can I use some different labware that I have on hand in the lab?

A: No, this protocol has been validated with specific labware. Any deviation from the specified plates and rack may result in incorrect pipetting or crashes. Contact technical support for further assistance if alternate labware is required (techserv@promega.com, techsupport@gilson.com).

Q: I noticed that one of the rows in the assay plate is a different color than the other rows. What could cause this?

A: From the Kinase Selectivity Profiling System Technical manual TM421, Figure 4:

"Each kinase family is identified by a unique strip tube color; the Kinase Strip ad corresponding Substrate/ Co-Factor Strip share the same strip tube color. To distinguish between the Kinase and Substrate/ Co-Factor Strips within the system, the Kinase Strips contain a blue dye and the Substrate/ Co-Factor Strips contain a yellow dye at the same position. The dye is always located in one of the top four tubes of the strip, and the position of the dye in an indication of the strip name"

Q: Can PIPETMAX accurately dispense 1 µL of compound per well?

A: Yes, PIPETMAX exceeds the Maximum Permissible Errors for piston operated multichannel pipettes (ISO 8655) at 1µL on the MAX8x20 pipette head:

		PIPETMAX MAX8x20	*ISO 8655*
1 µL	Systematic Error	±0.08 µL (8%)	±0.10 µL (10%)
1 µL	Random Error	±0.05 µL (5%)	±0.10 µL (10%)

Q: What are the recommended procedures for evaluating the data?

A: The Promega GloMax Luminometer is optimized for use with Promega Kinase Selectivity Profiling System and comes pre-loaded with protocols and settings for collecting data from the plates generated by PIPETMAX. If a GloMax is not available, data analysis worksheets can be found here: **http://www.promega.com/resources/tools/kinase-selectivity-profiling-systems-data-analysis-worksheets/**

Q: Why is ADP included in the kit?

A: ADP is provided with the Kinase Selectivity Profiling System kits for the optional manual preparation of a calibration curve using ADP and ATP.

Q: Why do the instructions specify 80 µM ATP instead of 100 µM ATP?

A: The automated protocol was found to work best with a larger volume of a more dilute form of ATP.

Q: How many tips are needed? Can I use the partial tip box from one run in a second run?

A: The single dose protocol, with two kinase strips and 20 compounds, requires two changes of tip boxes during the run. In this scenario, two full boxes plus a partial box of DS200ST are used (3 boxes), and six full boxes plus a partial box of DSL10ST are used (7 boxes). Partial tip boxes may be used in a subsequent run; enter the number of missing tips when prompted during Tip setup in the Step-by-step wizard.

Q: Do I always need to use two boxes of DSL10ST for the Dose Response Profile protocol?

A: When testing 1 kinase strip, 84 tips (DSL10ST) are consumed, which is less than one full box. It is possible to only use one box in this scenario, however the user will need to go through the Tip setup in the Step-by-step wizard to ensure that the instrument is aware that a box is missing.

Troubleshooting

Confirm that PIPETMAX is properly installed and aligned. Refer to *PIPETMAX® 268 User's Guide* for instructions.

Ensure that the removable tray and all plates, racks, and tips are properly seated on the instrument.

Remove tips from the tip waste bin during all incubation steps to prevent tip waste bin overfilling.

Ensure that all reagents are properly thawed and mixed prior to use.

Ensure there are no bubbles in the reagent source plate which could result in the aspiration of air instead of liquid.

Only Gilson 384 validated tips should be used with this protocol.

Check the 384-well plate before use—if it rocks when placed on the bench (i.e., warped and not level) choose a different plate.

For assistance with questions relating to the Kinase Selectivity Profiling System, and the automated protocols (KSPS Single Dose Profile, KSPS Dose Response Profile), contact Promega technical support (techserv@promega.com).

For assistance with questions relating to PIPETMAX system operation, contact Gilson technical support (techsupport@gilson.com).

Appendix

Footprint Dimensions for Microplates: ANSI SLAS 1-2004 (R2012):
https://www.slas.org/default/assets/File/ANSI_SLAS_1-2004_FootprintDimensions.pdf

Kinase Selectivity Profiling System Technical manual TM421: http://www.promega.com/resources/protocols/technical-manuals/101/kinase-selectivity-profiling-system-general-panel-protocol/

Video: Automation of the Kinase Selectivity Profiling Systems:
http://www.promega.com/resources/multimedia/cell-signaling/automation-of-the-kinase-selectivity-profiling-systems/

Custom Kinase Selectivity System Tool: http://www.promega.com/products/cell-signaling/kinase-assays/customize/

Kinase Selectivity Profiling Systems Data Analysis Worksheets:
http://www.promega.com/resources/tools/kinase-selectivity-profiling-systems-data-analysis-worksheets/