

## Methyltransferase ASSAY LAYOUT SHEET

for use with Assay Designs' Catalog No. 907-025

### End Point Assay Protocol Flow Chart:

Well ID:	Blank A1, A2	Enzyme Dilutions A3-A10	Positive Control A11, A12
Mix all reagents gently	---	---	---
Transferase Assay Buffer	25 $\mu$ L	---	---
Methyltransferase dilution	---	25 $\mu$ L	---
Spiked Reaction Mix	25 $\mu$ L	25 $\mu$ L	25 $\mu$ L
Detection Solution	100 $\mu$ L	100 $\mu$ L	100 $\mu$ L
Incubate 20 min. @ 25°C, sealed	⇒⇒⇒⇒	⇒⇒⇒⇒	---
Positive control	---	---	25 $\mu$ L
Incubate 10 min. @ 25°C, sealed	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Isopropyl Alcohol	50 $\mu$ L	50 $\mu$ L	50 $\mu$ L
Read Fluorescence at 380 <sub>ex</sub> /520 <sub>em</sub>	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒

### MT Inhibition Assay Protocol Flow Chart:

Well ID:	Blank B1, B2	Inhibition Samples B3-B10	D0 B11, B12
Mix all reagents gently	---	---	---
Transferase Assay Buffer	25 $\mu$ L	---	10 $\mu$ L
Inhibitor Dilution	---	10 $\mu$ L	---
Methyltransferase	---	15 $\mu$ L	15 $\mu$ L
Incubate 10 min. @ RT, sealed	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Spiked Reaction Mix	25 $\mu$ L	25 $\mu$ L	25 $\mu$ L
Detection Solution	100 $\mu$ L	100 $\mu$ L	100 $\mu$ L
Incubate 30 min. @ RT, shaking, sealed	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Isopropyl Alcohol	50 $\mu$ L	50 $\mu$ L	50 $\mu$ L
Read Fluorescence at 380 <sub>ex</sub> /520 <sub>em</sub>	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒

**Kinetic Assay Protocol Flow Chart:**

<b>Well ID:</b>	<b>Blank D1, D2</b>	<b>Enzyme Dilutions E1-H12</b>
Mix all reagents gently	---	---
Transferase Assay Buffer	25 µL	---
Methyltransferase Dilution	---	25 µL
Reaction Mix	25 µL	25 µL
Detection Solution	100 µL	100 µL
Incubate @ 25°C, sealed	⇒⇒⇒⇒	⇒⇒⇒⇒
Isopropyl Alcohol [Stop replicate dilutions at pre-determined time intervals]	50 µL	50 µL
Read Fluorescence at 380 <sub>ex</sub> /520 <sub>em</sub>	⇒⇒⇒⇒	⇒⇒⇒⇒

**Methyltransferase PLATE LAYOUT:**

A <sub>1</sub> Blank	A <sub>2</sub> Blank	A <sub>3</sub> Dil.1	A <sub>4</sub> Dil.1	A <sub>5</sub> Dil.2	A <sub>6</sub> Dil.2	A <sub>7</sub> Dil.3	A <sub>8</sub> Dil.3	A <sub>9</sub> Dil.4	A <sub>10</sub> Dil.4	A <sub>11</sub> Pos. Control	A <sub>12</sub> Pos. Control
B <sub>1</sub> Blank	B <sub>2</sub> Blank	B <sub>3</sub> Inhib. Dil.1	B <sub>4</sub> Inhib Dil.1	B <sub>5</sub> Inhib Dil. 2	B <sub>6</sub> Inhib Dil. 2	B <sub>7</sub> Inhib Dil. 3	B <sub>8</sub> Inhib Dil. 3	B <sub>9</sub> Inhib Dil. 4	B <sub>10</sub> Inhib Dil.4	B <sub>11</sub> D0	B <sub>12</sub> D0
C <sub>1</sub> Kinetic Blank	C <sub>2</sub> Kinetic Blank	C <sub>3</sub>	C <sub>4</sub>	C <sub>5</sub>	C <sub>6</sub>	C <sub>7</sub>	C <sub>8</sub>	C <sub>9</sub>	C <sub>10</sub>	C <sub>11</sub>	C <sub>12</sub>
D <sub>1</sub> Kinetic D1	D <sub>2</sub> Kinetic D1	D <sub>3</sub> Kinetic D1	D <sub>4</sub> Kinetic D1	D <sub>5</sub> Kinetic D1	D <sub>6</sub> Kinetic D1	D <sub>7</sub>	D <sub>8</sub>	D <sub>9</sub>	D <sub>10</sub>	D <sub>11</sub>	D <sub>12</sub>
E <sub>1</sub> Kinetic D2	E <sub>2</sub> Kinetic D2	E <sub>3</sub> Kinetic D2	E <sub>4</sub> Kinetic D2	E <sub>5</sub> Kinetic D2	E <sub>6</sub> Kinetic D2	E <sub>7</sub>	E <sub>8</sub>	E <sub>9</sub>	E <sub>10</sub>	E <sub>11</sub>	E <sub>12</sub>
F <sub>1</sub> Kinetic D3	F <sub>2</sub> Kinetic D3	F <sub>3</sub> Kinetic D3	F <sub>4</sub> Kinetic D3	F <sub>5</sub> Kinetic D3	F <sub>6</sub> Kinetic D3	F <sub>7</sub>	F <sub>8</sub>	F <sub>9</sub>	F <sub>10</sub>	F <sub>11</sub>	F <sub>12</sub>
G <sub>1</sub> Kinetic D4	G <sub>2</sub> Kinetic D4	G <sub>3</sub> Kinetic D4	G <sub>4</sub> Kinetic D4	G <sub>5</sub> Kinetic D4	G <sub>6</sub> Kinetic D4	G <sub>7</sub>	G <sub>8</sub>	G <sub>9</sub>	G <sub>10</sub>	G <sub>11</sub>	G <sub>12</sub>
H <sub>1</sub> Kinetic D5	H <sub>2</sub> Kinetic D5	H <sub>3</sub> Kinetic D5	H <sub>4</sub> Kinetic D5	H <sub>5</sub> Kinetic D5	H <sub>6</sub> Kinetic D5	H <sub>7</sub>	H <sub>8</sub>	H <sub>9</sub>	H <sub>10</sub>	H <sub>11</sub>	H <sub>12</sub>