

**Methyltransferase ASSAY LAYOUT SHEET**  
for use with Enzo Life Sciences Catalog No. ADI-907-025

**End Point Assay Protocol Flow Chart:**

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

**MT Inhibition Assay Protocol Flow Chart:**

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

## Kinetic Assay Protocol Flow Chart:

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

**Methyltransferase PLATE LAYOUT:**

A1 Blank	A2 Blank	A3 Dil.1	A4 Dil.1	A5 Dil.2	A6 Dil.2	A7 Dil.3	A8 Dil.3	A9 Dil.4	A10 Dil.4	A11 Pos. Control	A12 Pos. Control
B1 Blank	B2 Blank	B3 Inhib. Dil.1	B4 Inhib Dil.1	B5 Inhib Dil. 2	B6 Inhib Dil. 2	B7 Inhib Dil. 3	B8 Inhib Dil. 3	B9 Inhib Dil. 4	B10 Inhib Dil.4	B11 D0	B12 D0
C1 Kinetic Blank	C2 Kinetic Blank	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1 Kinetic D1	D2 Kinetic D1	D3 Kinetic D1	D4 Kinetic D1	D5 Kinetic D1	D6 Kinetic D1	D7	D8	D9	D10	D11	D12
E1 Kinetic D2	E2 Kinetic D2	E3 Kinetic D2	E4 Kinetic D2	E5 Kinetic D2	E6 Kinetic D2	E7	E8	E9	E10	E11	E12
F1 Kinetic D3	F2 Kinetic D3	F3 Kinetic D3	F4 Kinetic D3	F5 Kinetic D3	F6 Kinetic D3	F7	F8	F9	F10	F11	F12
G1 Kinetic D4	G2 Kinetic D4	G3 Kinetic D4	G4 Kinetic D4	G5 Kinetic D4	G6 Kinetic D4	G7	G8	G9	G10	G11	G12
H1 Kinetic D5	H2 Kinetic D5	H3 Kinetic D5	H4 Kinetic D5	H5 Kinetic D5	H6 Kinetic D5	H7	H8	H9	H10	H11	H12