EPIGENEOUS™
METHYLTRANSFERASE ASSAY 1,000 TESTS

PROTOCOL

For research use only.
Not for use in therapeutic or diagnostic procedures.

Storage temperature: ≤-60°C.

www.cisbio.com

Product information:
Document reference: 62SAHPEB rev03 (January 2020)

Packaging details:

	384-well small volume white plate (20 µL)
62SAHPEB	1,000 tests

1. Assay description

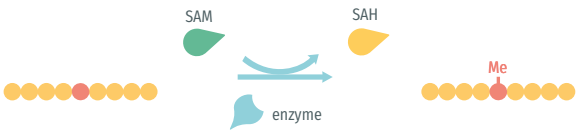
The EPIgeneous™ Methyltransferase assay is a universal biochemical assay for all enzymes that produce S-adenosylhomocysteine (SAH). This assay is a generic method for detecting SAH and can be used for any enzyme class that produces SAH.

The methyl group (CH3) attached to the methionine sulfur atom in S-Adenosyl methionine (SAM) is chemically reactive and allows donation of this group to an acceptor substrate in transmethylation reactions. Methyltransferase enzymes catalyse the transfer of a Methyl group from SAM to a substrate generating SAH.

The assay involves two main steps described below:

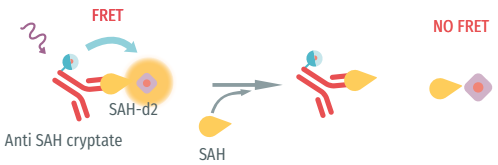
1.1. Enzymatic step:

During this step, the substrate is incubated with the enzyme in presence of compounds to be tested. SAM (CH3 donor) is added to start the enzymatic reaction leading to the substrate methylation.

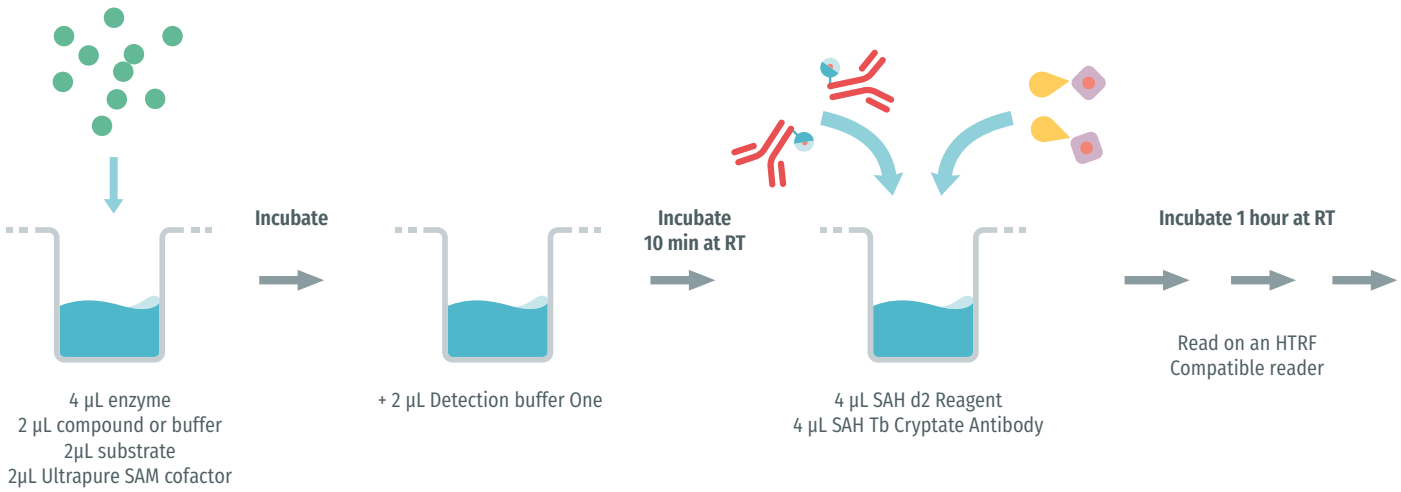


1.2. Universal detection step:

The detection method is based on a competitive immunoassay between native SAH and d2 coupled SAH towards an antibody specific to SAH labeled with Lumi4-Tb. The resulting TR-FRET signal is inversely proportional to the concentration of SAH in the standard or in the sample.



2. Protocol at a glance:



PerkinElmer
For the Better

3. HTRF reagents:

		Stock concentration	Number of vials	Volume per vial	Storage	Ref # (when available separately)
Ultrapure SAM Cofactor (340 nmol) * (S-(5' Adenosyl)-L-methionine)	green cap	2 mM	1	170 µL	≤-60°C	62SAHZLC
SAH Standard * * (S-(5' Adenosyl)-L-homocysteine)	purple cap	500µM	1	400 µL	≤-60°C	62SAHCLB
EPIgeneous Diluent Buffer	white cap	Ready-to-use	1	13 mL	≤-16°C	
EPIgeneous Detection Buffer One	transparent cap	Ready-to-use	2	1.2 mL	≤-60°C	
SAH d2 Reagent (Small) *	blue cap	See §5.4 (preparation of SAH-d2)	1	550 µL	≤-16°C	62SAHDLA
SAH Tb Cryptate Antibody 1,000 tests	Red cap	50X	1	90 µL	≤-16°C	
EPIgeneous Detection Buffer Two	Red cap	Ready-to-use	1	13 mL	≤-16°C	

* Amount of reagent provided is sufficient for 1,000 tests (and 5 standard curves) using 20µM SAM. For higher concentrations extra vials can be ordered separately

** Amount of reagent provided is sufficient for 5 standard curves at 200µM SAM/SAH. For higher concentrations or extra standard curves extra vials can be ordered separately

4. Storage stability:

All reagents should be kept frozen until used.

Avoid repeated freeze / thaw cycles.

After thawing, the stock solutions can be dispensed into single use aliquots and stored at recommmended tempteratures.

5. Reagent preparation

HTRF® reagent concentrations have been set for optimal assay performances. Note that any dilution or improper use will affect the assay's quality.

For an accurate quantitative determination of sample, calibration curves must be carried out with the enzymatic buffer used for the enzymatic step.

Be careful, working solution preparation may differ between the 1,000 and the 10,000 data point kits.

- Thaw all reagents at room temperature, allow them to warm up (caution: take buffers' thawing time into account)
- We recommend centrifuging the vials gently after thawing before pipeting the stock solutions
- Prepare the working solutions from stock solutions (§3) by following the instructions below

5.1. Preparation of working solutions for enzymatic step:

Depending on the enzyme, the enzymatic buffer (not provided) should be optimized.

Note that it is important to use Ultrapure SAM provided by Cisbio. Using other SAM sources can affect assay efficiency.

It is recommended to use concentrations of SAM between 0.4 µM and 200 µM (enzymatic step concentrations), for other conditions, please contact our technical support.

Note that concentrations of DTT over 1mM may affect the results by decreasing the efficiency of the detection step.

			Working solution	Volume dispensed	Final concentration (enzymatic step)
Compounds	**Not provided**	**Dilute with enzymatic buffer**	**5X**	**2µL**	**1X**
Substrate	**Not provided**	**Dilute with enzymatic buffer**	**5X**	**2µL**	**1X**
Enzyme	**Not provided**	**Dilute with enzymatic buffer**	**2.5X**	**4µL**	**1X**
SAM	**2 mM**	**Dilute with enzymatic buffer**	**5X**	**2µL**	**1X**

5.2. Detection Buffer One:

This reagent is ready to use.

Note that use of Detection Buffer One is mandatory and any dilution of the Detection Buffer One will impair results by decreasing the efficiency of the detection step.

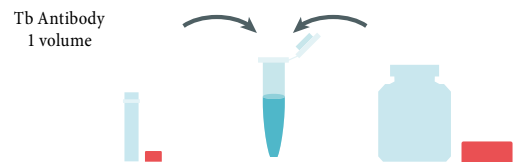
The Detection Buffer One is packaged in DMSO. Thaw and store it at room temperature for experiments. Do not put it on ice before dispensing as it will freeze.

5.3. Preparation of SAH Tb Cryptate Antibody:

Concentration has been determined for optimal assay performances. Note that any dilution or improper use of the Tb Cryptate Reagent will affect the assay's quality.

Prepare an SAH Tb Cryptate Antibody working solution with Detection Buffer Two. Refer to the image on the right:

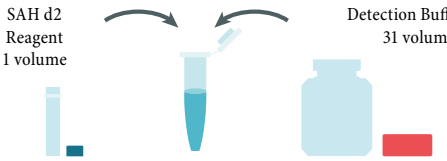
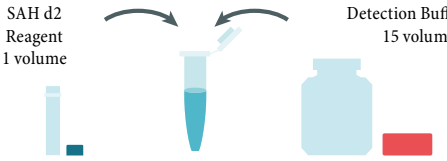
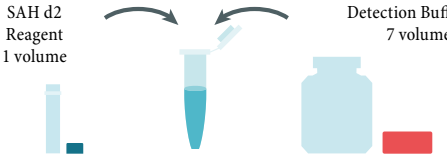
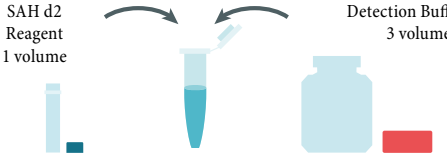
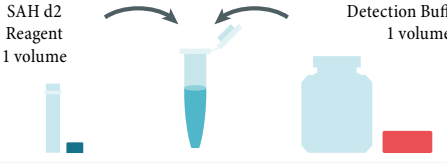
Anti-SAH-Lumi4-Tb conjugate



Dilute 50-fold the frozen Tb Cryptate Reagent stock solution with Detection Buffer Two:
e.g. add 90 µL of Tb Antibody stock to 4410 µL (4.41 mL) of Detection Buffer Two

5.4. Preparation of SAH d2 Reagent:

Caution! Concentration of SAH d2 Reagent depends on the concentration of SAM required for the assay. Dilute the SAH-d2 in Detection Buffer Two according to the following table:

[SAM] during the enzymatic step	SAH-d2 stock solution	SAH-d2 reagente	
0.4 µM ≤ SAM < 1 µM	50 X		**SAH d2 Reagent 50X:** Dilute 50-fold the frozen SAH d2 Reagent stock solution with Detection Buffer Two. e.g. add 100 µL of d2-reagent stock to 4900 µL (4.9 mL) of Detection Buffer Two.
1 µM ≤ SAM ≤ 2 µM	32 X		**SAH d2 Reagent 32X:** Dilute 32-fold the frozen SAH d2 Reagent stock solution with Detection Buffer Two. e.g. add 100 µL of d2-reagent stock to 3100 µL (3.10 mL) of Detection Buffer Two.
2 µM < SAM ≤ 10 µM	16 X		**SAH d2 Reagent 16X:** Dilute 16-fold the frozen SAH d2 Reagent stock solution with Detection Buffer Two. e.g. add 100 µL of d2-reagent stock to 1500 µL (1.5 mL) of Detection Buffer Two.
10 µM < SAM ≤ 20 µM	8 X		**SAH d2 Reagent 8X:** Dilute 8-fold the frozen SAH d2 Reagent stock solution with Detection Buffer Two. e.g. add 100 µL of d2-reagent stock to 700 µL (0.7 mL) of Detection Buffer Two.
20 µM < SAM < 100 µM	4 X		**SAH d2 Reagent 4X:** Dilute 4-fold the frozen SAH d2 Reagent stock solution with Detection Buffer Two. e.g. add 100 µL of d2-reagent stock to 300 µL (0.3 mL) of Detection Buffer Two.
100 µM ≤ SAM ≤ 200 µM	2 X		**SAH d2 Reagent 2X:** Dilute 2-fold the frozen SAH d2 Reagent stock solution with Detection Buffer Two. e.g. add 100 µL of d2-reagent stock to 100 µL (0.1 mL) of Detection Buffer Two.

5.5. SAM/SAH Standard Curve preparation:

The Standard curve mimics a methyltransferase reaction, as SAH is produced, SAM is depleted. The adenosyl (i.e. [SAM]+[SAH]) total concentration should remain constant throughout the range studied.

- A diluent (Tris HCl 50 mM pH 8.8, 0.01 % Tween20) that can be used as reference buffer for the standard curve preparation is provided in the kit, but depending on the enzyme, the enzymatic buffer has to be optimized.
- For an accurate quantitative determination of sample, calibration curves must be carried out with the enzymatic buffer used for the enzymatic step.
- Standard curves should be performed with the same concentration of SAM used for the assay.
- It is recommended to use concentrations of SAM between 0.4 µM and 200 µM, for other conditions, please contact our technical support.

Examples of standard curves for 1 µM and 20 µM SAM are shown below.

SAH Standard 8 solutions are prepared respectively at 1 µM and 20 µM then diluted in SAM solutions (1 µM and 20 µM) as shown below:

Standard curve for SAM 1 µM

				Working concentrations			
	Standard preparation			SAH (nM)	/	SAM (nM)	% SAM Conversion
Standard 8	150 µL SAH 1 µM			1,000	/	0	100
Standard 7	50 µL Standard 8	+	100 µL SAM 1 µM	333	/	667	33.3
Standard 6	50 µL Standard 7	+	100 µL SAM 1 µM	111	/	889	11.1
Standard 5	50 µL Standard 6	+	100 µL SAM 1 µM	37	/	963	3.7
Standard 4	50 µL Standard 5	+	100 µL SAM 1 µM	12.3	/	987.7	1.23
Standard 3	50 µL Standard 4	+	100 µL SAM 1 µM	4.1	/	995.9	0.41
Standard 2	50 µL Standard 3	+	100 µL SAM 1 µM	1.4	/	998.6	0.14
Standard 1	50 µL Standard 2	+	100 µL SAM 1 µM	0.5	/	999.5	0.05
Standard 0	100 µL SAM 1 µM			0	/	1,000	0

Standard curve for SAM 20 µM

				Working concentrations			
	Standard preparation			SAH (nM)	/	SAM (nM)	% SAM Conversion
Standard 8	150 µL SAH 20 µM			20,000	/	0	100
Standard 7	50 µL Standard 8	+	100 µL SAM 20 µM	6,667	/	13,333	33.3
Standard 6	50 µL Standard 7	+	100 µL SAM 20 µM	2,222	/	17,778	11.1
Standard 5	50 µL Standard 6	+	100 µL SAM 20 µM	741	/	19,259	3.7
Standard 4	50 µL Standard 5	+	100 µL SAM 20 µM	247	/	19,753	1.23
Standard 3	50 µL Standard 4	+	100 µL SAM 20 µM	82	/	19,918	0.41
Standard 2	50 µL Standard 3	+	100 µL SAM 20 µM	27	/	19,973	0.14
Standard 1	50 µL Standard 2	+	100 µL SAM 20 µM	9	/	19,991	0.05
Standard 0	100 µL SAM 20 µM			0	/	20,000	0

6. Detailed protocol

The enzymatic assay is performed as described below using different controls:

Negative Control (Std 0): 0% SAM Conversion (No Enzyme). This control consists of the anti-SAH antibody-Lumi4-Tb and SAH-d2 in the presence of enzymatic reaction components (without the enzyme) and SAM (0% SAH). This control defines the upper limit of the assay window. Appropriate negative control must be prepared for each SAM concentration tested.

Maximum HTRF Ratio Control: This control consists of the anti-SAH antibody-Lumi4-Tb and SAH-d2 with other reagents. You may have a different HTRF Ratio between this control and the Negative Control (Std 0). This is due to the antibody slight cross reactivity over SAM at high concentrations.

Buffer control: used to make sure that buffers are not contaminated by Cryptate and do not generate any background fluorescence.

Cryptate control: used to check the Cryptate signal at 620nm. This control defines the lower limit of the assay signal.

Use white plates.

Plates to be purchased separately, visit www.cisbio.com/microplates-recommendations for more information.

Do not premix SAH d2 Reagent and anti SAH Tb Cryptate Antibody conjugates as it will affect results by changing the kinetics of the detection (equilibrium not obtained at 1h incubation).

	Standard Curve		Enzymatic Assay		Blank controls	
	Maximum HTRF Ratio Control	Standard	Negative control	Sample	Cryptate control	Buffer control
Enzymatic step (10µL)						
Standard		10 µL				
Enzymatic buffer	10 µL		4 µL		10 µL	10 µL
Enzyme				4 µL		
Compound			2 µL	2 µL		
Substrate			2 µL	2 µL		
SAM			2 µL	2 µL		
Seal plate and incubate for enzymatic reaction						
Detection Step (20µL)						
Detection Buffer One	2 µL	2 µL	2 µL	2 µL	2 µL	2 µL
Seal plate and incubate 10 min at room temperature						
Detection Buffer Two					4 µL	8 µL
SAH d2 Reagent	4 µL	4 µL	4 µL	4 µL		
SAH Tb Crypate Antibody	4 µL	4 µL	4 µL	4 µL	4 µL	
Seal plate and incubate 1h at room temperature						

Remove plate sealer and read on an HTRF® compatible reader*

Check that you are using the set-up for Lumi4-Tb donor.

Using an inappropriate set-up may seriously affect the results.

* For information about HTRF® compatible readers and for set-up recommendations, please visit our website at: cisbio.com/compatible-readers

Substrate and SAM or enzyme and compound can be premixed if required.

7. Data reduction

The table below presents the results obtained with the standard protocol of 20µL.

The fluorescence is measured at 620 nm (Cryptate) and 665 nm (d2). Results are calculated from the 665nm/620nm HTRF® ratio.

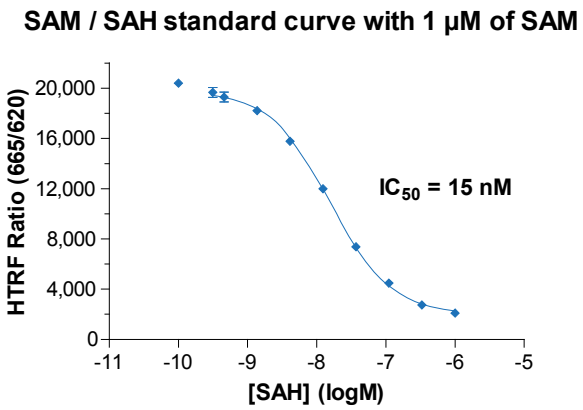
This data should be considered only as an example (readings on PHERAstarFS with flash lamp from BMG Labtech). Results may vary from one HTRF compatible reader to another.

SAM and SAH solutions have been diluted in the EPIgeneous Diluent Buffer (Tris HCl 50 mM pH 8.8, 0.01 % Tween20).

The curve is drawn by plotting HTRF® Ratio versus the [SAH] concentrations.

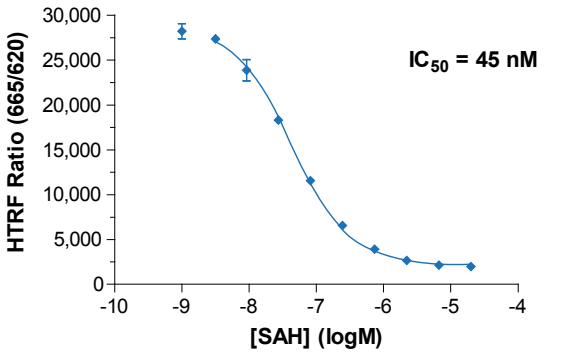
Example of standard curve for 1 µM SAM/SAH

		Blank (Cryptate control)			Ratio (1)	CV% (2)
	% SAM Conversion	SAH	/	SAM		
Maximum HTRF Ratio	0	0 nM	/	0 nM	20,397	1.5
Standard 0	0	0 nM	/	1,000 nM	19,657	2.0
Standard 1	0.05	0.5 nM	/	999.5 nM	19,297	1.7
Standard 2	0.14	1.4 nM	/	998.6 nM	18,222	1.1
Standard 3	0.41	4.1 nM	/	995.9 nM	15,781	0.1
Standard 4	1.23	12.3 nM	/	987.7 nM	12,005	1.8
Standard 5	3.7	37 nM	/	963 nM	7,372	1.7
Standard 6	11.1	111 nM	/	889 nM	4,474	2.6
Standard 7	33.3	333 nM	/	667 nM	2,735	0.5
Standard 8	100	1,000 nM	/	0 nM	2,094	6.3



Example of standard curve for 20 µM SAM/SAH

		Blank (Cryptate control)			Ratio (1)	CV% (2)
	% SAM Conversion	SAH	/	SAM		
Maximum HTRF Ratio	0	0 nM	/	0 nM	28,211	2.9
Standard 0	0	0 nM	/	20,000 nM	27,363	0.6
Standard 1	0.05	9 nM	/	19,991 nM	23,860	4.0
Standard 2	0.14	27 nM	/	19,973 nM	18,311	0.2
Standard 3	0.41	82 nM	/	19,918 nM	11,553	0.7
Standard 4	1.23	247 nM	/	19,753 nM	6,568	1.8
Standard 5	3.7	741 nM	/	19,259 nM	3,914	0.6
Standard 6	11.1	2,222 nM	/	17,778 nM	2,653	1.6
Standard 7	33.3	6,667 nM	/	13,333 nM	2,144	1.3
Standard 8	100	20,000 nM	/	0 nM	1,999	1.0



Ratio (1)	Signal 665nm / Signal 620nm x 10^4	Ratio must be calculated for each individual well.
CV% (2)	Standard deviation / Mean ratio x 100	The mean and standard deviation can then be worked out from ratio replicates.
For more information about data reduction, please visit our website at: cisbio.com/data-reduction		

To obtain additional information or support, please contact your technical support team (www.cisbio.com/contact-us)

FOR MORE INFORMATION
Europe and other countries +33(0)466-796-705 U.S. and Canada 1-888-963-4567 China +86-21-5018-9880
Japan +81-(0)43-306-8712 Visit www.cisbio.com to find a list of our regional distributors

PerkinElmer®
For the Better