

MANUAL

Technology: HTRF®

Epigenetics

HTRF Anti histone H3K27 Me3-Eu Cryptate mAb conjugate

Part number	61KC3KAE	61KC3KAD
Test size	500 tests	10,000 tests

Storage: $\leq -20^{\circ}\text{C}$

Version: 04

Date: February 2024

REAGENT FORMULATION

This Eu³⁺-cryptate labeled antibody is supplied in 50 mM HEPES buffer pH7.0, 0.1% protease free bovine serum albumine (BSA).

The recommended amount of antibody to be used per well is specified in the included product data sheet provided with each lot. Each vial supplies enough reagent to perform 500 tests (ref 61KC3KAE) or 10,000 tests (ref 61KC3KAD) in 384 well low volume plate with a 20µl final assay volume.

ANTIBODY SPECIFICITY

The antibody specificity was evaluated by performing titrations of methylated biotin-peptides.

Product	Cross-reactivity
H3(21-44)	0%
H3(21-44)K27me1	0.20%
H3(21-44)K27me2	3.30%
H3(21-44)K27me3	100%

REAGENT HANDLING

Preparation of the working solution

- Allow the stock solution to thaw at room temperature.
- We recommend centrifuging the vials gently after thawing before pipeting the stock solutions.
- Mix the solution gently by pipeting several times.
- Dilute the stock solution to the working concentration (refer to the product data sheet provided with the reagent). Mix gently.

In order to avoid repeated freeze and thaw cycles, stock solution can be divided into aliquots and frozen for additional use (according to storage conditions).

Recommended buffer

We recommend to use HTRF detection buffer 62SDBRDD for the preparation of the working solution.

The use of SDS should be avoided due to it's denaturing effect on XL665.

ASSAY FLEXIBILITY AND MINIATURIZATION

When used as suggested, one small size vial (ref 61KC3KAE) will provide sufficient reagent for 500 tests using a 384-well low volume plate in 20 µL final assay volume.

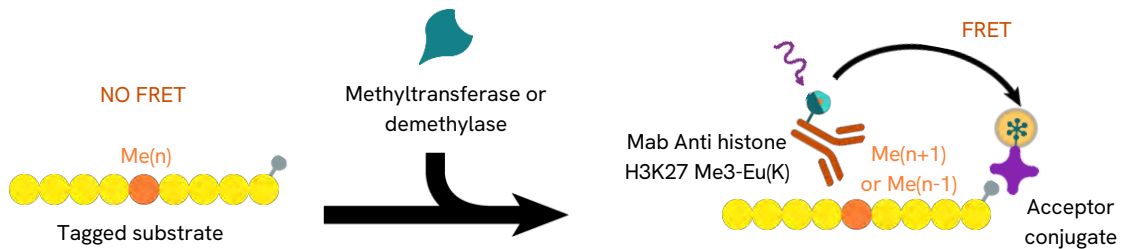
To change the plate formats (96 half-well or 1536-well) and final volumes (100 µL to less than 10 µL), the volume of each assay component is simply proportionally adjusted in order to maintain the reagent concentrations. For example, for a 1536 well plate in 10 µL volume, half the amount of material will be used per well allowing 1,000 tests to be run. Miniaturization will not affect assay performance.

Assay component	Volume proportion	Assay format		
		1536-well (10 µL)	384-well low volume (20 µL)	96 half-well (100 µL)
Other assay components	2 volumes	5 µL	10 µL	50 µL
XL665 conjugate	1 volume	2.5 µL	5 µL	25 µL
Cryptate conjugate	1 volume	2.5 µL	5 µL	25 µL
		1,000 tests	500 tests	100 tests

STORAGE CONDITIONS AND STABILITY

-20°C	Long term storage
	No more than one freeze / thaw cycle

ASSAY PRINCIPLE AND COMPANION REAGENTS



As illustrated above, all methyltransferase or demethylase assays are based on the same assay format. The enzymatic reaction is carried out with a biotinylated substrate. The methylated substrate is then detected using the specific anti-methyl antibody coupled to Eu³⁺-cryptate with streptavidin-XL665 (ref 610SAXLA).

If required, a [Lys(me3)27]-Histone H3 (21-44)-(biotin) peptide can be used as positive control to check HTRF signal appearance with detection conjugates. This peptide can be purchased from Anaspec #64367. Follow the provider recommendations for reconstitution and storage.

REACH European regulations and compliance

This product contains material of biologic origin. Use for research purposes only. Do not use in humans or for diagnostic purposes. The purchaser assumes all risk and responsibility concerning reception, handling and storage.



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www.revvity.com

revvity

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com

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