

# Product Information



## JMJD2A Tudor Domains Ligand/APC Acceptor Mixture

Item No. 700962

### IMPORTANT

Water used to prepare all reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for TR-FRET. UltraPure water may also be purchased (Item No. 400000).

### Laboratory Procedures

#### 420-well vial JMJD2A Tudor Domains Ligand/APC Acceptor Mixture (384- or 1,920-well kit):

For each 384-well plate, add 2.1 ml of 1X TR-FRET Assay Buffer 2 to one vial of the JMJD2A Tudor Domains Ligand/APC Acceptor Mixture (420 wells, Item No. 700962) and gently vortex. Keep the solution in the dark to prevent photobleaching. Long-term storage of the diluted mixture is not recommended.

OR

#### 2,100-well vial JMJD2A Tudor Domains Ligand/APC Acceptor Mixture (9,600-well kit):

For five 384-well plates, add 3 ml of 1X TR-FRET Assay Buffer 2 to one vial of the JMJD2A Tudor Domains Ligand/APC Acceptor Mixture (2,100 wells, Item No. 700962) and gently vortex. Transfer contents to a new tube and adjust the mixture to a final volume of 10.5 ml in 1X Assay Buffer. Keep the solution in the dark to prevent photobleaching. Long-term storage of the diluted mixture is not recommended.

### Suggested Assay Protocol

This product has been tested and formulated to work exclusively with the JMJD2A Tudor Domains Inhibitor Screening Assay Kit (Item No. 700960). This product may not perform as described if used with other assay reagent or protocols. Follow the steps below to accurately measure the TR-FRET ratio in the assay. Allow all reagents except for the JMJD2A Tudor Domains Europium Chelate to equilibrate to room temperature prior to performing the assay. Keep the JMJD2A Tudor Domains Europium Chelate on ice until just prior to use. Volumes indicated below are for a 384-well plate format with a 20  $\mu$ L final assay volume.

#### 1. Inhibitor Samples

Dilute inhibitor samples in 1X TR-FRET Assay Buffer 2 to a concentration that is 4X the desired final concentration (e.g. if 1  $\mu$ M is desired, prepare a 4  $\mu$ M solution). This solution may contain up to 4% of an organic solvent (e.g. DMSO). Add 5  $\mu$ L of this dilution to the desired wells. For best results, perform the assay in duplicate.

It is recommended that inhibitor compounds be tested in a concentration-response format with at least eight independent concentrations that span approximately a 1,000-fold range around the expected IC<sub>50</sub> value of the inhibitor.

#### 2. Positive and Negative Control Samples

For positive (inhibitor control) control wells, add 5  $\mu$ L of JMJD2A positive control to the desired wells.

For negative (no inhibition) control wells, add 5  $\mu$ L of 1X TR-FRET Assay Buffer 2 to the desired wells. If inhibitor samples from step 1 contain organic solvent, add an equivalent amount of the solvent into the assay in this step.

#### 3. JMJD2A Tudor Domains Europium Chelate

Add 10  $\mu$ L of the diluted JMJD2A Tudor Domains Europium Chelate to every well of the 384-well plate.

#### 4. Pre-incubation (optional)

If desired, incubate the control and sample wells for 15 minutes at room temperature to allow pre-equilibration of the inhibitor and control compounds with the JMJD2A Tudor Domains Europium Chelate. *Protect from light.*

#### 5. JMJD2A Tudor Domains Ligand/APC Acceptor Mixture

Add 5  $\mu$ L of the reconstituted JMJD2A Tudor Domains Ligand/APC Acceptor Mixture to every well.

**WARNING: THIS PRODUCT IS FOR LABORATORY RESEARCH ONLY. NOT FOR ADMINISTRATION TO HUMANS. NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.**

#### SAFETY DATA

This material should be considered hazardous until information to the contrary becomes available. Do not ingest, swallow, or inhale. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling. This information contains some, but not all, of the information required for the safe and proper use of this material. Before use, the user must review the complete Safety Data Sheet, which has been sent via email to your institution.

#### WARRANTY AND LIMITATION OF REMEDY

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery.

Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence.

This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a **refund** of the purchase price, or at Cayman's option, the **replacement**, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our **Warranty and Limitation of Remedy located on our website and in our catalog.**

Copyright Cayman Chemical Company, 03/11/2014

### Cayman Chemical

#### Mailing address

1180 E. Ellsworth Road  
Ann Arbor, MI  
48108 USA

#### Phone

(800) 364-9897  
(734) 971-3335

#### Fax

(734) 971-3640

#### E-Mail

custserv@caymanchem.com

#### Web

www.caymanchem.com

# Product Information



## 6. Incubation

Seal the plate with an adhesive aluminum seal and incubate at room temperature for one hour. For automation purposes, the plate does not have to be sealed, but it should remain in the dark to prevent photobleaching.

## 7. Reading the Plate

Read the plate(s) in time-resolved format by exciting the sample at 340 nm and reading emissions at 620 and 670 nm, using a 100  $\mu$ s delay and a 500  $\mu$ s read window. To ensure optimal assay sensitivity, it is strongly recommended that a filter-based instrument be used to perform TR-FRET measurements. The plate reader used at Cayman Chemical employs a 340/40 nm excitation filter, 620/15 nm, and 670/20 nm emission filters. Samples will be stable for analysis for at least five hours if stored at room temperature and protected from light. Data analysis is performed using the TR-FRET ratio (670 nm emission/620 nm emission).

## Related Product

For a list of related products please visit: [www.caymanchem.com/catalog/700962](http://www.caymanchem.com/catalog/700962)

## Cayman Chemical

### Mailing address

1180 E. Ellsworth Road  
Ann Arbor, MI  
48108 USA

### Phone

(800) 364-9897  
(734) 971-3335

### Fax

(734) 971-3640

### E-Mail

[custserv@caymanchem.com](mailto:custserv@caymanchem.com)

### Web

[www.caymanchem.com](http://www.caymanchem.com)