Aurora Biolabs
High Purity Protein for Research

eIF4E/eIF4G Binding Assay Kit

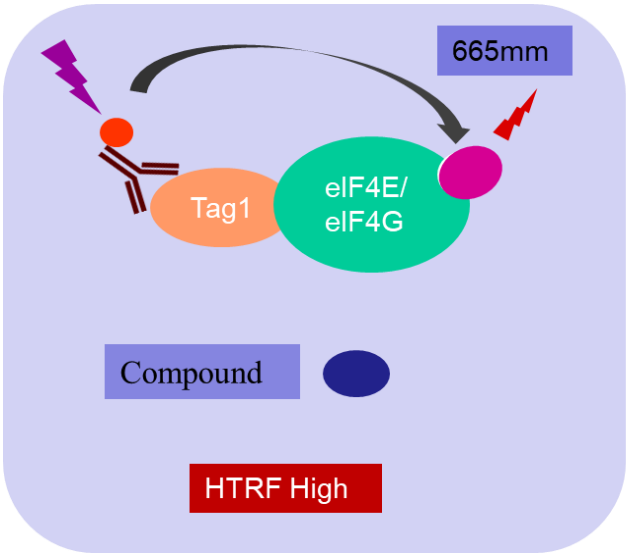
Catalog Number: 34343-BK

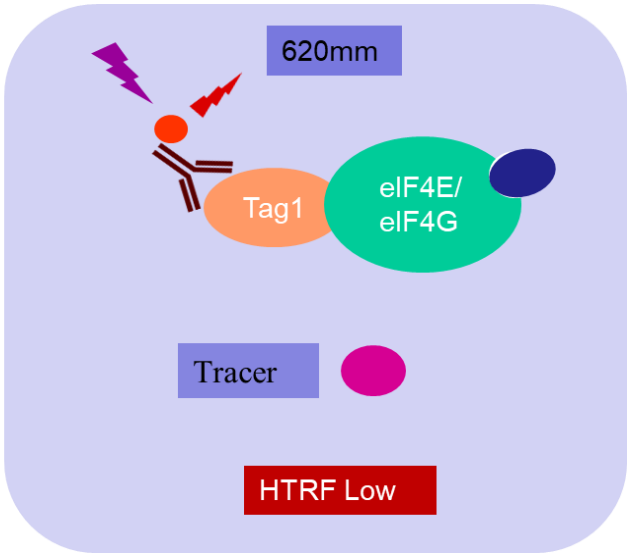
Background

Eukaryotic translation initiation factor eIF4E, the mRNA cap-binding protein, is considered the rate-limiting factor in translation. It plays an important role in cap-dependent translation initiation and recruitment of mRNA to ribosomes. Overexpression of eIF4E has been documented in numerous human cancers and contributes to transformation, tumorigenesis, and progression of cancers. Therefore, eIF4E is an attractive drug target for cancer treatment.

Assay Principle

The eIF4E binding assay kit, a TR-FRET based assay, is designed to screen compounds that bind to eIF4E. A fluorescence-labelled tracer and the N-terminal tagged full-length human eIF4E/eIF4G complex are used in this assay kit. A Terbium-labeled antibody binding to the tag on eIF4E serves as a fluorescence donor (HTRF donor). The binding of the fluorescence-labeled tracer to the eIF4E brings Terbium on the anti-Tag antibody close to the fluorophore on the tracer (HTRF acceptor). Activation of the Terbium results in fluorescence resonance energy transfer (FRET). Thus, the binding status can be quantitively measured by calculating the ratio of the emission fluorescence intensity of the acceptor (665 nm) and donor (620 nm). The competitive binding of a non-fluorescence compound will reduce the FRET signal.





Application

High throughput screening of compounds that bind to eIF4E/eIF4G.

Plate Reader

A HTRF® certified microplate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET) is required.

Components

Catalog number	Item	Amount	Storage
34343-BK-B	eIF4E Binding Assay buffer	25 mL	-20°C
34343-T1	Human eIF4E/eIF4G complex, Tag1	16 µL	-80°C
44782	Terbium-labeled anti-Tag1 antibody	10 µL	-80°C
67437	fluorescence-labeled tracer-M7-GDP (1 µM)	16 µL	-80°C
	384-well microplate, White	1	Room temperature

Materials needed but not supplied

1. Microplate reader, HTRF® certified microplate reader
2. 0.5 M DTT
3. Adjustable micro-pipettor
4. Sterile Tips

Assay protocol

1. Prepare assay buffer containing 1 mM DTT

   For example, mix 998 µl of assay Buffer and 2 µl of 0.5 M DTT. Make only enough DTT-containing assay buffer as needed for the assay. Store the remaining assay buffer at -20°C.

2. Prepare the inhibitor compound solution

   If the inhibitor compound is dissolved in water, make a solution of the compound 10-fold higher than the final concentration in 1X assay buffer (since you will add 2 µl to the 20 µl reaction).

   If the inhibitor compound is dissolved in DMSO, make a 100-fold higher concentration of the compound than the highest concentration you want to test in DMSO. Then make a 10-fold dilution in 1X assay buffer (at this step, the compound concentration is 10-fold higher than the final concentration and the DMSO concentration is 10%). To determine an IC50 or to test lower concentrations of the compound, prepare as series of further dilutions in 1X assay buffer containing 10% DMSO (the final concentration of the DMSO will be 1% in all samples).

3. Prepare eIF4E/eIF4G solution

   Thaw eIF4E/eIF4G protein on ice. Upon first thaw, briefly spin tube to recover the full contents at the bottom of the tube. Make aliquots of the enzyme for single use. Store remaining undiluted protein at -80°C.

   Note: eIF4E/eIF4G protein is sensitive to freeze/thaw cycles. Limit number freeze-thaw cycles for best results. Do not re-use the diluted protein.

   Dilute the eIF4E/eIF4G protein 200-fold (1 µL eIF4E/eIF4G + 199 µL 1X assay buffer containing DTT).

   Add 8 µl of diluted protein solution to each positive control wells and inhibitor test wells.

   Add 8 µl of 1X DTT containing buffer to each of negative control wells.

4. Add inhibitor

   Add 2 µl of diluted compound solution to each inhibitor test well.

   Add 2 µl of inhibitor solvent solution to each negative and positive control wells.

   Incubate at room temperature for 30 minutes (optional).

5. Prepare fluorescence-labeled tracer

   Thaw the tracer at room temperature.

   Dilute the tracer 125-fold (1 µL of 1 µM tracer + 124 µL 1X assay buffer containing DTT).

   Add 5 µl of diluted tracer to each well.

Dilute just enough tracer for the use. Store remaining undiluted tracer at -80°C. Do not re-use the diluted tracer.

6. Prepare dye solution

   Dilute Terbium-labeled anti-Tag antibody 1:200 (1 µl of Terbium-labeled anti-Tag antibody + 199 µl of 1X DTT-containing assay buffer).

   Add 5 µl of this dye mixture to each well.

   Dilute just enough of the antibody for each reaction set. Store remaining undiluted antibody at -80°C. Do not re-use the diluted antibody.

7. Incubate the reaction at room temperature for 60 minutes.

8. Measure fluorescent intensity

   HTRF compatible microplate reader is needed to measure fluorescent intensity of the samples. Fluorescent intensity should be measured twice:

   1. Excitation wavelength at 340 nm and emission at 620 nm.
   2. Excitation wavelength at 340 nm and emission at 665 nm.

Protocol Summary

Component	Negative Control	Positive Control	Inhibitor Test
1X buffer	8 µl		
eIF4E/eIF4G		8 µl	8 µl
Inhibitor solvent	2 µl	2 µl	
Inhibitor solution			2 µl
Subtotal Volume	**10 µl**	**10 µl**	**10 µl**
Incubate at room temperature for 30 minutes (Optional).			
Fluorescence-labeled tracer	5 µl	5 µl	5 µl
Dye solution	5 µl	5 µl	5 µl
Total Volume	**20 µl**	**20 µl**	**20 µl**
Incubate at room temperature for 60 minutes.			

Data Analysis

1. Calculate the ratio of the fluorescent intensity of each well.

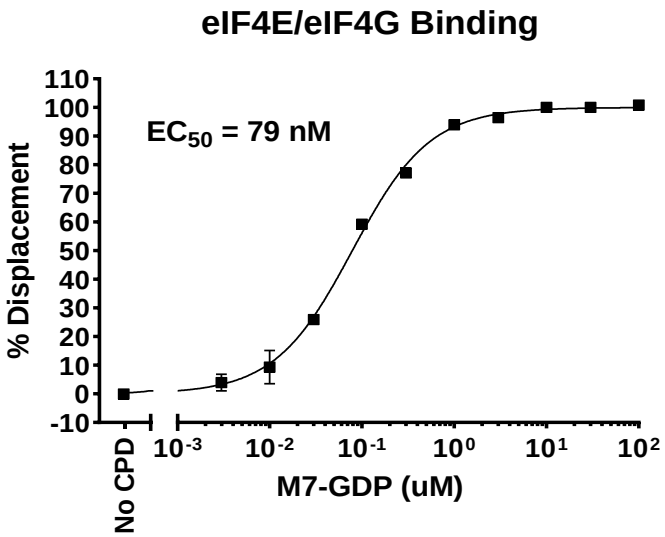
$$Ratio1 = \frac{\text{Fluorescent intensity at 665 nm}}{\text{Fluorescent intensity at 620 nm}}$$

2. Calculate percentage activity

In the absence of the compound (positive control), the sample signal (P) is defined as 100% activity. In the absence of enzyme (negative control), the sample signal (N) is defined as 0% activity. The percent activity in the presence of each compound is calculated according to the following equation: % activity = (S-N)/(P-N) X100, where S= the sample signal in the presence of the compound.

$$\% \text{ Displacement} = (1 - (\frac{S - N}{\text{P} - \text{N}}))X100$$

Assay result



Related products:

Product Name	Catalog #	Size
Kras Wild Type (WT), GST-tag	5727-4121G	50 µg, 100 µg
Kras WT, GST-tag, GDP Loaded	5727-WTG-G	50 µg, 100 µg
Kras WT, GST-tag, GppNHp loaded	5727-WTG-GP	50 µg, 100 µg
Kras G12C, His -tag	5727-4122H	50 µg, 100 µg
Kras G12C, GST-tag	5727-4122G	50 µg, 100 µg
Kras G12C, GST-tag, GDP Loaded	5727-4122G -G	50 µg, 100 µg
Kras G12C, GST-tag, GppNHp loaded	5727-4122G -GP	50 µg, 100 µg
Kras G12D, GST-tag	5727-4123G	50 µg, 100 µg
Kras G12D, GST-tag, GDP Loaded	5727-4123G -G	50 µg, 100 µg
Kras G12D, GST-tag, GppNHp loaded	5727-4123G -GP	50 µg, 100 µg

Kras G12R, GST-tag,	5727-4127G	50 µg, 100 µg
Kras G12R, GST-tag, GDP Loaded	5727-4127G -G	50 µg, 100 µg
Kras G12R, GST-tag, GppNHp loaded	5727-4127G -GP	50 µg, 100 µg
Kras G12V, GST-tag,	5727-4128G	50 µg, 100 µg
Kras G12V, GST-tag, GDP Loaded	5727-4128G -G	50 µg, 100 µg
Kras G12V, GST-tag, GppNHp loaded	5727-4128G -GP	50 µg, 100 µg
Kras WT Nucleotide Exchange Assay Kit	5727-4121NK	384 reactions
Kras G12C Nucleotide Exchange Assay Kit	5727-4122NK	384 reactions
Kras G12D Nucleotide Exchange Assay Kit	5727-4123NK	384 reactions
Kras G12R Nucleotide Exchange Assay Kit	5727-4173NK	384 reactions
Kras G12V Nucleotide Exchange Assay Kit	5727-4128NK	384 reactions
Kras WT – cRAF Binding Assay Kit	5727-4121BK	384 reactions
Kras G12C – cRAF Binding Assay Kit	5727-4122BK	384 reactions
Kras G12D– cRAF Binding Assay Kit	5727-4123BK	384 reactions
Kras G12V – cRAF Binding Assay Kit	5727-4128BK	384 reactions
Human RBD-RAF1, N-His tag, C-FLAG tag	7237231	50 µg, 100 µg
Human SOS1, No tag	7671	50 µg, 100 µg
Human SOS1, Avi-His tag	7671HA	50 µg, 100 µg
TEV-His	190001-R	1,000 Units, 10,000 Units
Recombinant SUMO Protease (Ulp1)	190003	1,000 units, 10,000 units
PreScission Protease (HRV 3C)	190002-R	1,000 units, 10,000 units
Recombinant YopH	200100	10 ug, 20 ug, 100 ug, 1 mg
Recombinant Mouse Leukemia Inhibitory Factor	11-0001	10 ug, 100 ug
Recombinant Human LIF	12-0002	10 ug, 100 ug, 1 mg
Recombinant Human LIF, Animal-Free	12-0002AFR	10 ug, 100 ug, 1 mg
Recombinant Human FGF-basic, Carrier-free	12-0005CFR	50 ug, 100 ug, 500 ug, 1 mg
Recombinant Human BCL2	225201	100 ug

Products are for research use only and are not intended for human use. We do not sell to patients.