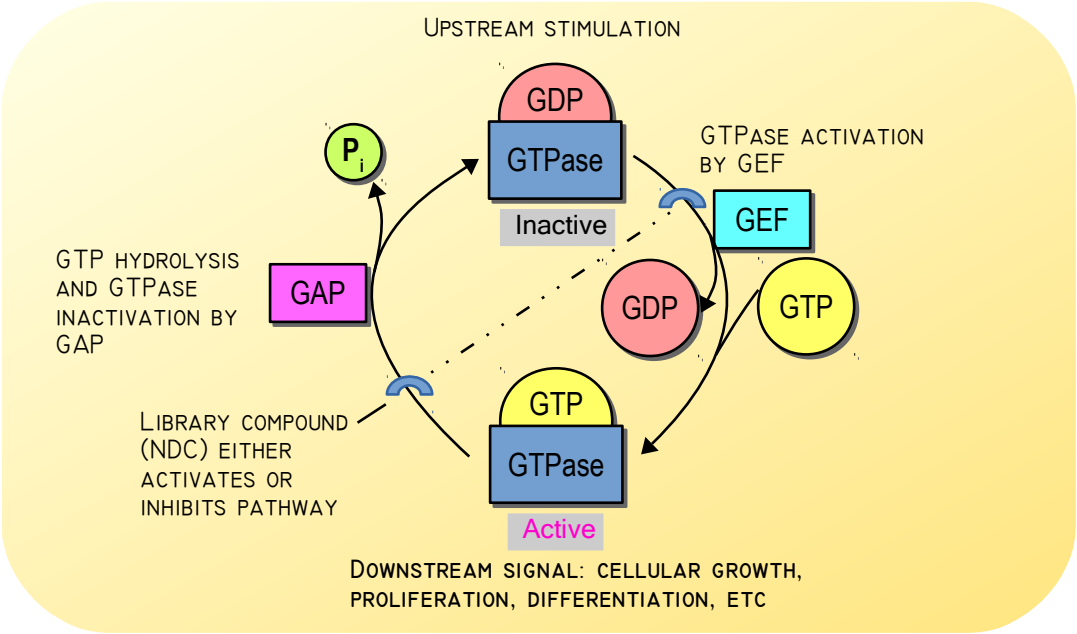
Oncogene & Cell Signalling Screening Kits

Single label homogeneous TRF modulation assay based on qFM TRF[1]

Multiple small GTPase measurements from one kit !

- GTP association and dissociation measurement for GEF
- Kinetic and endpoint readouts of association and dissociation
- A GTP Hydrolysis measurement for GAP pathways
- Determination of residual GTP from GAP pathways



BN Products & Services offer two homogeneous assays for measuring GTP pathways for GEF mediated GTP exchange and GTP hydrolysis based on residual concentration monitoring using a GTP specific antibody. The assays enable screening of possible candidate inhibitors for both GTP association and hydrolysis with small and large GTPases. The GTPases act as a “molecular switch”, shifting between GDP bound (inactive) and GTP bound (active) conformations. The cycle is controlled by guanine nucleotide exchange factor (GEF), and GTPase activating protein (GAP).

Researchers can screen the full GTP cycle effecting small molecule inhibitors by specifically monitoring GTP levels.

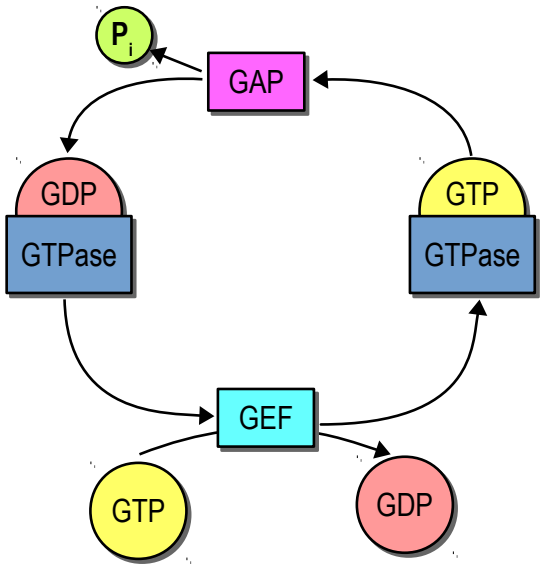
[1] Hemmilä I, Malminen O, Mikola H, Lövgren T, Clin Chem 34; 2320-2322 (1988)

BN Products & Services
www.bnpands.com

In the qFM TRF (fluorescent modulation time-resolved fluorescence) assay, the free Eu-GTP fluorescence emission is modulated when no hydrolysis occurs or when Eu-GTP is bound to GTPase. Hydrolysis reduces the amount of free GTP enabling Eu-GTP-antibody complex formation with resultant increased Eu-signal due to the bound Eu-chelate being protected from the modulator. Eu-GTP association to GTPase mediated by GEF also provides similar chelate protection and increased specific Eu-chelate signal

A new kit measuring both GTP pathways

Small GTPtase GEF GTP association and GTP Hydrolysis Assays

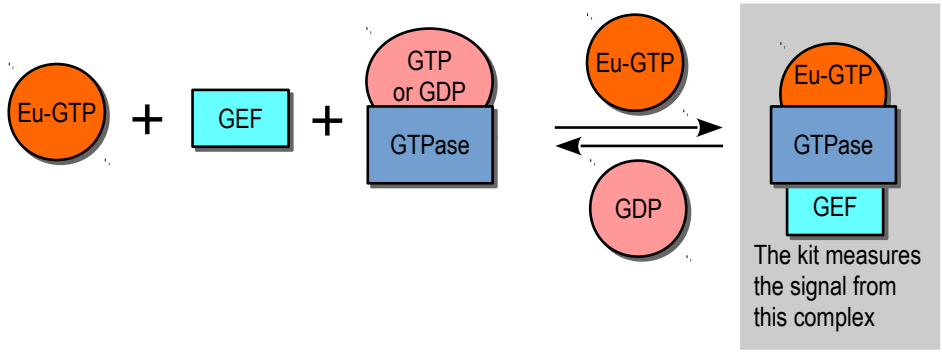


Rational behind the qFM TRF Screening Kits:

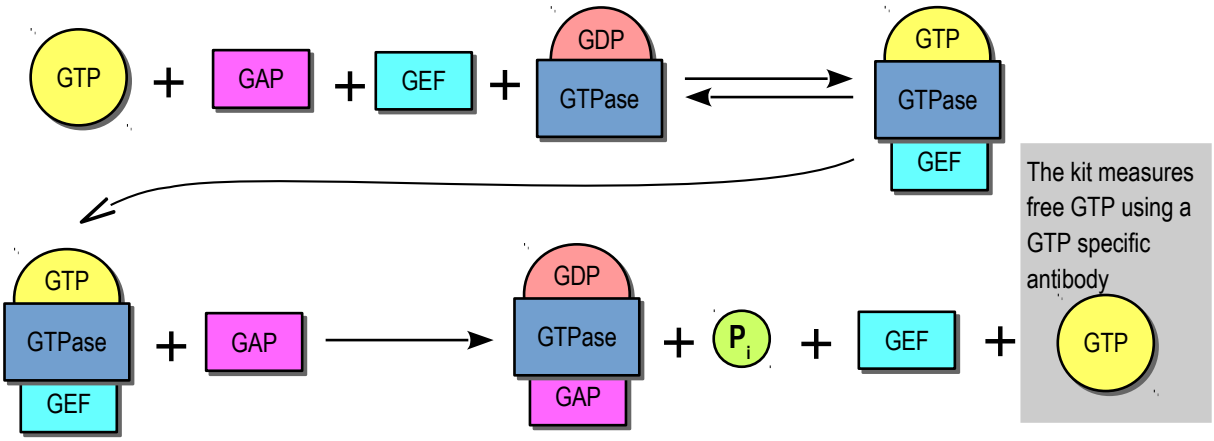
The modulated energy transfer (qFM TRF) based GTP detection is optimised to screen possible inhibitors for GTP association and hydrolysis with small and large GTPases. GTPases act as a “molecular switches”, shifting between GDP bound (inactive) and GTP bound (active) conformations. The cycle is controlled by guanine nucleotide exchange factor (GEF), and GTPase activating protein (GAP).

In the assay, the Eu-GTP is modulated when no hydrolysis occurs and unmodified GTP occupies the GTP antibody. Hydrolysis reduces the amount of free GTP enabling Eu-GTP-antibody complex formation and increased specific Eu-signal due to Eu-chelate protection from the modulator through binding to the free chelate and thus effectively removing the possible free label emission signal. This method was utilised to select inhibitor that influence H-Ras^{Wt} in either SOS^{cat} catalysed nucleotide exchange or p120GAP catalysed hydrolysis state.

Ras oncogene was discovered over four decades ago and is one of the main targets in oncology drug discovery with the ambition to discover effective treatments. The small G-proteins like Ras proteins function as binary switches circulating between GDP (inactive) and GTP(active)conformation. The switch function is controlled by the guanosine nucleotide exchange factor (GEF) and the GTPase-activating protein (GAP).



<u>GTP Association Assay</u>. The assay kit has simple to follow instructions for both an end point and kinetic read out of your screen.



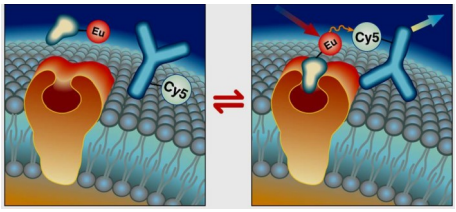
<u>GTP Hydrolysis Assay</u>. The assay kit has optimised instructions enabling the measurement of GTP concentration both end point and kinetically.

The primary function of the GEF proteins are to enable GTP association by dissociating the GDP from the GTPases. This process turns the GTPase proteins in the active state. The activated GTPase can interact with downstream effector proteins controlling key cellular processes in the cell. Mutated GTPase proteins are frequently found in cancer and other diseases, and most often they are locking in the GTP bound active state. This active state can be in the healthy state terminated by GAP protein catalysed GTP hydrolysis to GDP.

These qFM TRF Assays measure small and large GTPase activation and their activation kinetics are monitored in high throughput compatible 384-well plate format.

The kit contains the reagents required for labelling proteins and peptides with europium chelate for inclusion into either homogenous or enhanced separation based time-resolved fluorescence (TRF) assays.

TR FRET (Time-Resolved Fluorescence Resonance Energy Transfer) uses two fluorophore labels, in close proximity, a Eu chelate donor and a fluorescent acceptor. TR-FRET takes advantage of the long decay Eu donor to improve the performance of the FRET energy transfer ie the acceptor is activated to emit light at its given wavelength typically these are between 570-740nm.



qFM TRF assays (Fluorescence Modulation timeresolved fluorescence) only requires one labelled moiety , typically a Eu-chelate attached to a ligand. A proprietary assay buffer modulates any free chelate fluorescence enabling direct measurement of only the bound ligand-receptor or ligand-cell complex. Both end point and kinetic assays can be constructed. (Hemmilä et al, 1985)

Separation-based TReF (Time-resolved enhanced fluorescence Assays) - requires a coated plate or other solid phase to bind and separate one component of the assay - ie antibody, oligonucleotide, fusion tags, PNA , soluble receptors, cell mono- layer, etc. The reaction is stopped and all free label removed through a wash step. The bound Eu-complex fluorescence is measured after a dissociative enhancement step: by addition after the wash step, of a second chelating reagent, to recapture all Eu from the bound component and reform a new highly fluorescent chelate.

Multipled TReF assays (Eu, Sm, Tb and or Tb) can be designed for routine screening by exploiting the spectral and temporal differences between lanthanides. (Described and published by Hemmilä et al, 1979.)

Distributed by:

Product Information

Labelling Kits
Kit contents:
- Lyophilized Europium chelate
- Europium standard
- Enhancement solution for separation assays
- Full instructions

E41000 QuickAllAssay Labelling Kit,W1024 1mg
E40200 QuickAllAssay Labelling Kit, W1024 200ug
E40100 QuickAllAssay Labelling Kit,W1024 100ug
E4000A QuickAllAssay Labelling Kit, for Academics
Activated DTPA (Eu, Eu & Sm, or Eu,Sm & Tb) Kits

Activated Chelates (ITC,DTA etc)
CE1000 QuickAll Assay TEKES chelate (activated),1mg
CE0500 QuickAllAssay TEKES chelate (activated), 500ug
SC0500 W8044 chelate activated for homogeneous assays in high EDTA & salt environments 100ug - 500ug
Activated DTPA chelates (Eu, Sm & Tb) ie ITC, IAA, Malemide or DTA

Dissociative Enhancement Solution for TreF assays
E51000 Enhancement Solution, 1 Litre
E50250 Enhancement Solution, 250ml
E50050 Enhancement Solution, 50ml

Labelled antibodies and tags (contact us for full listing)
Eu-labelled Streptavidin
Eu-labelled anti-GST Antibody
Eu-Labelled anti-Histag antibody
Eu-labelled anti-FLAG antibody
Eu-labelled anti-streptavidin
Eu-labelled anti animal antibodies - contact us for listing

Homogeneous assay Kits
Q10001 GTP Association Assay Kit 2 x384 well plates
Q81001 GTP Hydrolysis & Association Assay 2 x 384 plates
Q90010 GTP Association Assay 10 plates
Q90020 GTP Association Assay 20 plates
Q82005 GTP Association & Hydrolysis Assay 5 plates
Q20005 Estrodiol immunoassay 5 plates

Full assay development, ELISA to TRF assay conversion and biomolecule custom labeling services

Contact us for optimal FRET acceptor listing

Products&Services
http://www.bnpands.com (BP Products & Services)
http://www.bionobile.com (Bio-Nobile™ products)
Email: info@bnpands.com Tel. +358-40-8359271
Turku, Finland