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# ab270553
# GTPase Assay Kit

## A product of Expedeon, an Abcam company

Applicable to Expedeon product codes: 602-0120

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GTPase Assay Kit - datasheet:
www.abcam.com/ab270553
(use www.abcam.cn/ab270553 for China, or www.abcam.co.jp/ab270553 for Japan)

For measuring the activity of GTPase enzymes in microplates.

This product is for research use only and is not intended for diagnostic use.

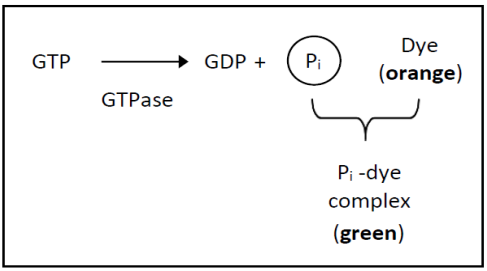
# Table of Contents



## 1. Overview

GTPase Assay Kit - (ab270553) employs a 96-well plate format with all the reagents necessary for measuring GTPase activity. The kit also contains PiColorLock™ (a superior malachite green reagent) which has special additives to prevent backgrounds arising out of non-enzymatic GTP hydrolysis The PiColorLock™ assay is based on the change in absorbance of the dye malachite green in the presence of phosphomolybdate complexes. Unlike most malachite dye-based solutions,

PiColorLock™ gives a stable end- point signal and is not prone to precipitation. PiColorLock™ has special additives to prevent backgrounds arising from nonenzymatic GTP hydrolysis.




Principle of the GTPase assay

## 2. Materials Supplied and Storage

Store kit at 4°C immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Avoid repeated freeze-thaws of reagents.

| Item | 192 assays | Storage temperature (before prep) |
|---|---|---|
| PiColorLock™ | 1 x 10 mL | 4°C |
| Accelerator | 1 x 0.25 mL | 4°C |
| Stabilizer | 1 x 5 mL | 4°C |
| 0.1 M Magnesium chloride | 1 x 1.5 mL | 4°C |
| 0.5 M Tris pH 7.5 | 1 x 5 mL | 4°C |
| 0.1 mM Pi Standard | 1x 5 mL | 4°C |
| GTP (lyophilized) | 4 vials | -20°C or -70°C |
| 96-well plates | 2 plates | RT |

***Δ Note:*** *Caution - PiColorLock™ is very acidic.*

# 3. Technical Considerations

## 3.1 Checking for Pi contamination

Since the assay measures Pi released from GTP, any free Pi present in the assay components before the reaction starts will give rise to a background signal. The most common source of free Pi is the enzyme sample itself.

***Δ Note:*** *All crude extracts of tissues and cells will contain free phosphate unless steps have first been taken to remove it.*

To check if your enzyme sample contains free Pi, mix the assay components shown in the table, below.

| Solution | Enzyme (µL) | MilliQ water (µL) | 0.1 mM Pi (µL) |
|---|---|---|---|
| 1 | 100 | 100 | 0 |
| 2 | 100 | 60 | 40 |
| 3 | 0 | 160 | 40 |
| 4 | 0 | 200 | 0 |

***Δ Note:*** *Ideally 2 and 3 will give high signals and 1 and 4 will give low signals. If the signal for sample 1 is relatively high compared with that for sample 4, your enzyme is contaminated. Free Pi in the enzyme may be eliminated by dialysis or desalting.*

***Δ Note:*** *Phosphate buffer should not be used to prepare tissue or cell homogenates.*

To remove free Pi from the sample, please check the Inorganic Phosphate Binding Resin (ab270547).

## 3.2 Effects of some common substances:

| Component | Concentration* | Effect |
|---|---|---|
| NaCl | 250 mM | None |
| KCl | 250 mM | None |
| $MgCl_2$ | 25 mM | None |
| DTT | 0.25 mM | Slight signal loss |
| β-ME | 0.5 mM | None |
| Tris | 25 mM | None |
| HEPES | 25 mM | None |
| MES | 25 mM | None |
| MOPS | 25 mM | None |
| BSA | 0.1 mg/mL | None |
| BSA | 1.0 mg/mL | Risk of precipitation |
| DMSO | 2.5% | None |
| Detergents | 0.03% | See below |

***Δ Note:*** **The stated values refer to concentrations in the assay samples before the addition of PiColorLock™ mix.*

***Δ Note: ***** *Very low concentrations of detergent (0.002-0.005%) may cause precipitation. If a detergent is needed, use a concentration of >0.03%. Tween 20 is usually a good choice.*

## 3.3 How to determine assay conditions:

There are no strict guidelines and the key parameters (i.e. enzyme dilution, assay time and temperature) will depend on the intrinsic activity of the particular enzyme, the concentration of the enzyme, and operator preferences with regard to assay time and temperature. The enzyme sample and GTP are usually incubated for between 15 and 30 mins before addition of the Pi-detection reagent; very short assay times with very concentrated enzyme should be avoided. Plate assays are more easily performed at room temperature than at 37°C. It is important to operate in the 'linear range' of the assay i.e. under conditions where doubling or halving of the amount of enzyme gives twice or half of the original signal, respectively. The simplest way to determine the linear range is to select your preferred assay time (e.g. 30 mins) and temperature (e.g. 23°C), which leaves only one further parameter to be

investigated, the dilution of enzyme. The linear range is evident from a plot of absorbance versus enzyme dilution. If the absorbance value of any sample exceeds 2 it is usually necessary to dilute the enzyme further.

### 3.4 Blanks

In general, you should set up assay blanks (i.e. lacking enzyme) and subtract the average blank value from all other wells before you do any calculations with the assay data e.g. calculating the amount of phosphate generated by reference to the standard curve.

## 4. Reagent Preparation

### 4.1 GTP substrate:

Reconstitute the lyophilized GTP to 10 mM concentration by adding the volume of MQ $H_2O$ stated on the vial label. Keep the GTP on ice when not in use and snap freeze any surplus reagent (aliquot to avoid multiple freeze-thaw cycles) and store at -70˚C.
***Δ Note:*** *The reconstitution volume is batch specific. Please contact our technical Support team for details.*

### 4.2 Substrate/ buffer mix:

The assay kit is supplied with 0.5 M Tris assay buffer pH 7.5, but you can substitute any other non-phosphate-containing buffer to suit your particular GTPase. All GTPases will require a metal ion cofactor, often Mg2+ (0.1 M $MgCl_2$ is supplied with the kit) but again you can substitute your own metal ion, or include other metal ions, as required.

The table below shows the volumes required to make up substrate/buffer mix for selected numbers of wells. The GTP concentration in this mix is 1 mM. Once the enzyme has been added to the substrate/ buffer mix, the final buffer, $Mg^{2+}$ and GTP concentrations are 50 mM, 2.5 mM, and 0.5 mM, respectively.

| No. of wells | 0.5 M Buffer (µL) | 0.1 M $MgCl_2$ (µL) | 10 mM GTP (µL) | MilliQ water (µL) |
|---|---|---|---|---|
| 1 | 20 | 5 | 10 | 65 |
| 25 | 500 | 125 | 250 | 1,625 |
| 50 | 1,000 | 250 | 500 | 3,250 |
| 75 | 1,500 | 375 | 750 | 4,875 |
| 100 | 2,000 | 500 | 1,000 | 6,500 |
| 150 | 3,000 | 750 | 1,500 | 9,750 |
| 200 | 4,000 | 1,000 | 2,000 | 13,000 |
| 250 | 5,000 | 1,250 | 2,500 | 16,250 |
| 500 | 10,000 | 2,500 | 5,000 | 32,500 |

## 4.3 PiColorLock™ mix:

Prepare the mix immediately before the reagent is required by adding 1/100 volume of Accelerator to PiColorLock™ .

The table below gives the volumes of reagent required for the specified numbers of wells.

| No. of wells | PiColorLock™ (µL) | Accelerator (µL) |
|---|---|---|
| 1 | 50 | 0.5 |
| 25 | 1,250 | 12.5 |
| 50 | 2,500 | 25 |
| 75 | 3,750 | 37.5 |
| 100 | 5,000 | 50 |
| 150 | 7,500 | 75 |
| 200 | 10,000 | 100 |
| 250 | 12,500 | 125 |
| 500 | 25,000 | 250 |

***Δ Note:*** *The PiColorLock™ mix cannot be stored for long periods; make up only what you will use on the day.*

## 5. Assay procedure

– Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.

**5.1** Make substrate/buffer mix.

**5.2** Make PiColorLock™ mix by adding Accelerator to PiColorLock™ reagent just before use.

**5.3** Set up assays with your GTPase; add 100 µL enzyme to 100 µL substrate/buffer mix.

**5.4** Incubate for a fixed time at set temperature.

**5.5** Add 50 µl of PiColorLock™ mix to stop reactions.

**5.6** After 2 mins, add 20 µl of Stabilizer and mix thoroughly by pipetting up and down.

**5.7** After 30 mins, read the plate at a wavelength in the range 590- 660nm.

# 6. Standard Curve

## 6.1 Preparation of standard curve:

A standard curve is required if you wish to calculate enzyme activity.

- Prepare a set of Pi standards using the 0.1 mM Pi stock (see the table, below).
- Set up duplicate wells containing 200 µL of each standard and add 50 µL of PiColorLock™ mix.
- Two minutes later add 20µL of Stabilizer, and mix thoroughly.
- After 30 mins, read the plate. Subtract the blank values (i.e. for sample 12) and plot absorbance versus concentration of Pi.

| Tube # | 0.1 mM Pi Standard (µL) | MilliQ water (µL) | Concentration of Pi (µM) |
|---|---|---|---|
| 1 | 500 | 500 | 50 |
| 2 | 450 | 550 | 45 |
| 3 | 400 | 600 | 40 |
| 4 | 350 | 650 | 35 |
| 5 | 300 | 700 | 30 |
| 6 | 250 | 750 | 25 |
| 7 | 200 | 800 | 20 |
| 8 | 150 | 850 | 15 |
| 9 | 100 | 900 | 10 |
| 10 | 50 | 950 | 5 |
| 11 | 25 | 975 | 2.5 |
| 12 | 0 | 1,000 | 0 |

## 7. Enzyme Activity Calculation:

If you need to do this, one unit is the amount of enzyme that catalyzes the reaction of 1 µmol of substrate per minute. The activity (units/ml) of your undiluted enzyme sample is given by the equation:

$$\text{Activity} = (AxC)/500B$$

Where:

A = concentration of Pi (µM) determined from the standard curve (see Section 6).

B = assay time is in minutes.

C = enzyme dilution factor (Note: the value for 'C' must be 1 or >1; e.g. C= 100 for a 1/100 dilution of enzyme).

***Δ Note:*** *The above equation is valid only if the assay is set up as indicated in the protocol (i.e. the assay volume is 200 µl, comprising 100 µl of enzyme and 100 µl of substrate mix).*

## 8. Typical Data

Data provided **for demonstration purposes only**.

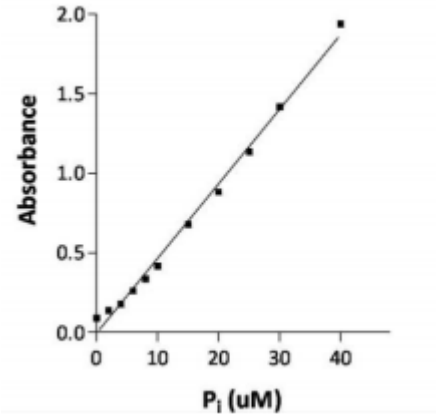



**Figure 1:** Standard Curve for High Throughput GTPase Assay Kit - PiColorLock™

# 9. FAQ

### 1. Why do I get a high background when my enzyme definitely has no free Pi?

This is almost certainly caused by inadequate mixing of the stabilizer. This results in a high background signal because of non-enzymatic decay of GTP substrate. The stabilizer is added in a relatively small volume (20 µL), and the operation of pipetting up and down with a pipette set to 20 µL volume may not result in sufficient mixing when the total volume is 270 µL. Try pipetting up and down while stirring at the same time. Alternatively, add the stabilizer with one pipette set at 20 µL volume and mix using a larger pipette set to ~150 µL volume. This ensures thorough mixing of the stabilizer solution with minimal effort.

### 2. I would like to measure the conversion of pyrophosphate to phosphate. Can I use the PiColorLock™ Phsophate Detection System for this purpose?

Yes, only the phosphate will give a signal, pyrophosphate will not.

### 3. I have 5% DMSO in my assay. Can I use PiColorLock™?

Yes, the reagent is designed for drug screening work and other situations that require DMSO.

### 4. I have phosphate in my enzyme. What can I do?

You can dialyse or desalt the enzyme into a phosphate-free buffer. Alternatively, you can use the Inorganic Phosphate Binding Resin (ab270547) resin to remove the phosphate.

## Technical Support



**For all technical or commercial enquiries please go to:**

www.abcam.com/contactus

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