

**ab120687**

**Pitstop 2™**

## Instructions for Use

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

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# 1. Introduction

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Pitstop 2™ directly binds to the clathrin terminal domain at a site that overlaps with that of clathrin box containing accessory protein ligands (von Kleist et al, 2011). In the case of amphiphysin 1 the IC50 value is about 12 µM. However, this may vary for other proteins, typically between 10 and 60 µM. This is a medium-to-low affinity compound.

Pitstop 2™ is an amphiphile, which allows the compound to penetrate cell membranes. As with all amphiphiles, it is advisable not to overdose the application of the drug as this will likely lead to strong side-effects. Thus users are advised to follow the protocol provided.

## 2. Storage and Handling

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- A stock solution should be prepared by dissolving in 100% DMSO (fresh, sterile) to give a final concentration of 30 mM. Vortexing should be used to ensure solubilization of the compound. This stock solution is stable at room temperature for about 4-6 hrs.
- Aliquots of the stock solution should be stored at -20°C. Stock solutions should not be left at room temperature for longer than necessary to prepare the working solutions. Avoid freeze/thaw cycles of the stock solution as this may affect the activity of the compound.
- The final working concentration of DMSO should generally be between 0.3 and 1%. Low concentrations of DMSO (i.e. 0.1%) may lead the compound to precipitate out of solution. However, please note that the amount of DMSO needed to keep the compound in solution is dependent on the concentration of the compound. For example, in 1% DMSO, drug concentrations of 300 µM or higher may also lead to precipitation (depending on which aqueous buffer is used and the temperature).

### 3. Preparation of Working Solutions

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- Pitstop 2™ can be directly diluted in aqueous media to the final working concentration and is compatible with a range of buffers.
- Pitstop 2™ and the Pitstop 2™ negative control are not water soluble, but can be used in low concentrations of DMSO (up to 1% for cellular experiments, up to 3% for in vitro use, see above and below) after serial dilution from the 100% DMSO stock solution.
- Clathrin-mediated endocytosis (CME) is a highly cooperative process; complete inhibition of CME at about 20-25  $\mu\text{M}$  Pitstop 2™ in most cell types has been observed. A final working concentration of 25  $\mu\text{M}$  is therefore recommended. Most cell lines will tolerate a concentration of 30  $\mu\text{M}$  Pitstop 2™ but primary cells generally tend to be more sensitive and could suffer from non-specific damage.
- The effect of storage and the re-use of the working solutions after dilution in aqueous buffers has not been investigated and is not recommended.

## 4. Guidelines for *in vitro* use

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1. For enzymatic experiments, a 1 - 3% DMSO final concentration is possible as, in the experience of the inventors of Pitstop 2™, 3% DMSO is the maximum tolerable concentration for most enzymes. However, for best results, it is recommended to always perform a DMSO dose titration to determine the maximum tolerable amount of DMSO for the enzyme of interest. After this amount of DMSO is determined, use this amount (or a sub maximum amount) to solubilise the compound
2. For cell experiments cells should be grown to at least 80-90% confluency on matrigel or poly-lysine coated supports. The media should then be removed and a serum-free media used.

Pitstop 2™ is amphiphilic and, like many small molecules, is sequestered by serum albumins. 0.1 - 0.2% serum can be tolerated for some cell lines, however this has not been extensively investigated. To the cells, add the working solution of Pitstop 2™ dissolved in buffer or serum free media (containing 10 mM HEPES; pH 7.4) to give a final concentration of 25 µM (in up to 1% DMSO). Incubate for 5 - 10 minutes, typically at 37°C. This incubation period should be sufficient to completely block CME of transferrin and other ligands, although the required time may depend on the cell line and ligand

Longer incubation times (such as greater than 30 minutes) may lead to non-specific effects and are therefore not recommended. Use of final concentrations of 30  $\mu$ M or above may occasionally lead to cells lifting from the plates (certain cell lines).

Additionally, high concentrations of Pitstop 2™ may interfere with fluorescence imaging due to a low emittance of the compound in the green channel. This fluorescence is not usually detectable if the cells have been first fixed and washed prior to imaging.

Special note when using electrically excitable cells, e.g. neurons: Neurons are particularly sensitive to amphiphilic compounds and may respond by firing action potentials. A final concentration of 15  $\mu$ M Pitstop 2™ is sufficient to completely block compensatory endocytosis at the presynaptic compartment.

A great advantage of Pitstop 2™ is that its effects on endocytosis are reversible. Incubation of Pitstop 2™-treated cells for 45-60 min (with two changes of media) in full serum containing medium will restore the ability of cells to undergo CME. This is an important control experiment that may be included in all experimental trials.

**For technical questions please do not hesitate to contact us by email ([technical@abcam.com](mailto:technical@abcam.com)) or phone (select “*contact us*” on [www.abcam.com](http://www.abcam.com) for the phone number for your region).**

## **Technical Support**

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