

## Certificate

Name of chemical: azidoblebbistatin

(S)-1-(4-Azido-phenyl)-3a-hydroxy-6-methyl-1,2,3,3a-tetrahydro-pyrrolo[2,3-b]quinolin-4-one

Cat. No: DR-A-081

M.w: 333.35

Extinction coeff at 427 nm:  $6100 \text{ M}^{-1}\text{cm}^{-1}$

Chemical purity: >95%

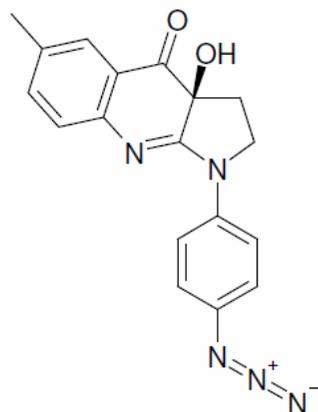
Ratio of (-) enantiomer: >99.5%

Storage conditions: in powder form or dissolved in DMSO in aliquots at  $-20 \text{ }^{\circ}\text{C}$  to limit freezing-thawing cycles. Always store in the dark.

NOTE: Complete darkness is usually not required when handling the compound. Normally no significant degradation occurs if azidoblebbistatin is shaded properly from direct light, e.g. during the day, if the lights are switched off in the room and no direct sunlight enters the room.

Solubility in water based buffers:  $\sim 7.5 \text{ }\mu\text{M}$  with 0.1%DMSO

Reference: **Azidoblebbistatin, a photoreactive myosin inhibitor** by Miklós Képiró, Boglárka H. Várkuti, Andrea Bodor, György Hegyi, László Drahos, Mihály Kovács, András Málnási-Csizmadia in *Proc Natl Acad Sci U S A*. 2012 Jun 12; 109(24):9402-7. doi: 10.1073/pnas.1202786109



## Recommendations for usage

1. Photoactivation: Recommended wavelength for initiating covalent crosslinking is around 300 nm.

2. Molecular tattoo: the irradiation parameters have to be optimized for the sample and the two-photon instrument.

General recommendations: The suggested wavelength for 2-photon irradiation is 860 nm. Based on the dimensions of the 2-photon laser focus, suggested step size is  $0.1 \text{ }\mu\text{m}/\text{pixel}$ . In most cases fast scanning is recommended. It is important to check how much irradiation the sample tolerates without drug treatment to avoid photodamage.

Example:

A protocol for the full inhibition of myosin in the stress fiber of a HeLa cell

drug concentration and incubation	5 $\mu\text{M}$ azidoblebbistatin, 15 mins
irradiated area (ROI)	2.4 $\mu\text{m}$ x 10-15 $\mu\text{m}$
irradiation wavelength	860 nm
laser intensity	3.5 % ( $\sim 20 \text{ mW}$ in the focal spot)
scanning speed	166.1 $\mu\text{m}/\text{ms}$
step size	0.1 $\mu\text{m}/\text{pixel}$
irradiation time	10 cycles repeated every 2 sec: scanning the ROI for 500 ms

3. Control experiments: when planning non-photoreactive control treatments, consider para-nitroblebbistatin as a control compound.

Reference: **Para-nitroblebbistatin, the non-cytotoxic and photostable myosin II inhibitor** by Miklós Képiró, Boglárka H. Várkuti, László Végner, Gergely Vörös, György Hegyi, Máté Varga, András Málnási-Csizmadia in *Angew Chem Int Ed Engl*. 2014 Jul 28;53(31):8211-5. doi: 10.1002/anie.201403540