Product Data Sheet

Product Name: CypK
Cat. No.: GC68928

Chemical Properties

Cas. No.	1610703-09-7		
Formula	$C_{12}H_{20}N_2O_4$	M.Wt	256.3
Solubility	DMSO : 30 mg/mL (117.05 mM; ultrasonic and adjust pH to 2 with HCl)	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 ℃ and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20℃ for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		
Structure	CypK		

Background

CypK (N-Cyclopropene-L-Lysine), a cyclopropene derivative of lysine, is efficiently incorporated into antibodies through genetic-code expansion. CypK is a minimal bioorthogonal handle for the creation of stable therapeutic protein conjugates[1][2].

Guidelines (Following is our recommended protocol. This protocol only provides a guideline, and should be modified according to your specific needs).

CypK assay[1] (express the antibody):

1. Thaw a vial of HEK suspension cells in a 250 mL flask containing 50 mL of expression medium supplemented with 100 units/mL penicillin, 100 µg/mL streptomycin, and 250 ng/mL amphotericin B. Keep the cells at 37 ℃ with 8% CO_2 in humidified incubators equipped with a shaker at 125 rpm. Split cells to 0.3-0.5 x 10^6 cells/mL (every 2-3 days) at least 2 times before transfecting.
2. When a density of 2.5 x 10^6 cells/mL is reached (2-3 days after splitting), prepare a fresh solution of 100 mM CypK. For this purpose, weigh 64 mg of CypK, add 2.5 mL of 0.1 sodium droxide, vortex, spin down to recover all undissolved particles and sonicate.
3. Add 2.5 mL of CypK (100 mM in 0.1 M NaOH) to 42.5 mL of expression medium

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supplemented with antibiotics. Mix well, add 250 µL of 0.1 M HCl, and sterilize using a 0.22 µm filter.
4.Dilute 50 µg of HC and LC pKym1 plasmids to 2.5 mL with reduced serum medium. In a separate tube, dilute 135 µL of transfection reagent to 2.5 mL with reduced serum medium.
5. Five minutes after preparing the solutions, mix the plasmids and the transfection reagent solution and incubate for 20 min to allow the formation of complexes between the DNA and the transfection reagent.
6. In the meantime, centrifuge 125 million cells at the target density for 5 min at 500 x g, resuspend with the expression medium containing CypK and add the DNA-transfection reagent mixture.
7. After incubating cells for 20 h, add 250 µL of transfection reagent enhancers included in the kit.
8. Harvest antibodies from the supernatant 6-7 days after addition of CypK (no change of medium is required during expression).

[1]. Oller-Salvia B. Genetic Encoding of a Non-Canonical Amino Acid for the Generation of Antibody-Drug Conjugates Through a Fast Bioorthogonal Reaction. J Vis Exp. 2018 Sep 14;(139):58066.
[2]. Oller-Salvia B, et, al. Rapid and Efficient Generation of Stable Antibody-Drug Conjugates via an Encoded Cyclopropene and an Inverse-Electron-Demand Diels-Alder Reaction. Angew Chem Int Ed Engl. 2018 Mar 5;57(11):2831-2834.