

产品名称: **VX-809 (Lumacaftor)**
 产品别名: **Lumacaftor; 鲁玛卡托; VRT 826809**

生物活性:					
Description	Lumacaftor (VX-809; VRT 826809) is a CFTR modulator that corrects the folding and trafficking of CFTR protein.				
IC ₅₀ & Target	EC50: 0.1 μM (CFTR)[1]				
In Vitro	In fischer rat thyroid (FRT) cells, Lumacaftor improves F508del-CFTR maturation by 7.1±0.3 fold (n=3) compared with vehicle-treated cells (EC ₅₀ , 0.1±0.1 μM; n=3) and enhances F508del-CFTR-mediated chloride transport by approximately fivefold (EC ₅₀ , 0.5±0.1 μM; n=3). At Lumacaftor concentrations greater than 10 μM, the response is reduced, resulting in a bell-shaped dose-response relationship with an IC ₅₀ of approximately 100 μM. Lumacaftor is orally bioavailable in rats and achieved in vivo plasma levels significantly above concentrations required for in vitro efficacy[1].Lumacaftor produces a concentration-dependent increase in the HRP luminescence signal after incubation with cells at 37°C or 27°C in both cell lines, with a similar EC ₅₀ value of approximately 0.3 μM. In F508-HRP CFBE41o ⁻ cells at 37°C, Lumacaftor increases the signal maximally to approximately 250 luminescence arbitrary units (a.u.) over the DMSO control baseline of approximately 60 a.u., representing an approximately 4-fold signal increase. Similarly, with the R1070W-HRP CFBE41o ⁻ cells, Lumacaftor increases the signal maximally to approximately 220 a.u. over the DMSO control baseline of approximately 85 a.u., representing an approximately 2.5-fold signal increase. Therefore, both cell lines produced robust signals with a good dynamic range for high-throughput screening [2].				
In Vivo	Oral dosing of 1 mg/kg Lumacaftor in male Sprague-Dawley rats results in a C _{max} of 2.4±1.3 μM with a t _{1/2} of 7.7±0.4 h (mean±SD; n=3), indicating that that Lumacaftor is orally bioavailable and able to reach plasma levels that significantly exceeded EC ₅₀ s for F508del-CFTR correction ^[1] .				
Solvent&Solubility	In Vitro: DMSO : 50 mg/mL (110.52 mM; Need ultrasonic)				
		Solvent Concentration Mass	1 mg	5 mg	10 mg
	Preparing	1 mM	2.2104 mL	11.0519 mL	22.1038 mL
	Stock Solutions	5 mM	0.4421 mL	2.2104 mL	4.4208 mL
		10 mM	0.2210 mL	1.1052 mL	2.2104 mL
	*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液；一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。				
	储备液的保存方式和期限：-80℃，6 months; -20℃，1 month。 -80℃ 储存时，请在 6 个月内使用，-20℃ 储存时，请在 1 个月内使用。				
In Vivo: 请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 In Vitro 方式配制澄清的储备液，再依次添加助溶剂： ——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶					
1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline Solubility: ≥ 3 mg/mL (6.63 mM); Clear solution 此方案可获得 ≥ 3 mg/mL (6.63 mM, 饱和度未知) 的澄清溶液。					

	以 1 mL 工作液为例, 取 100 μL 30.0 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中, 混合均匀向上述体系中加入 50 μL Tween-80, 混合均匀; 然后继续加入 450 μL 生理盐水定容至 1 mL。 2.请依序添加每种溶剂: 10% DMSO $\rightarrow$90% corn oil Solubility: $\geq$ 3 mg/mL (6.63 mM); Clear solution 此方案可获得 $\geq$ 3 mg/mL (6.63 mM, 饱和度未知) 的澄清溶液, 此方案不适用于实验周期在半个月以上的实验。 以 1 mL 工作液为例, 取 100 μL 30.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中, 混合均匀。
References	[1]. Van Goor F, et al. Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809. <i>Proc Natl Acad Sci U S A</i>. 2011 Nov 15;108(46):18843-8. [2]. Phuan PW, et al. Synergy-based small-molecule screen using a human lung epithelial cell line yields ΔF508-CFTR correctors that augment VX-809 maximal efficacy. <i>Mol Pharmacol</i>. 2014 Jul;86(1):42-51.
实验参考:	
Cell Assay	.A549 cells expressing F508-CFTR YFP are grown at 37°C/5% CO₂ for 18-24 hours after plating. The cells are then incubated with 100 μL of medium containing test compounds for 18-24 hours. At the time of the assay, cells are washed with PBS and then incubated for 10 minutes with PBS containing forskolin (20 μM) and genistein (50 μM). Each well is assayed individually for I⁻ influx by recording fluorescence continuously (200 milliseconds per point) for 2 seconds (baseline) and then for 12 seconds after rapid addition of 165 μL PBS in which 137 mM Cl⁻ is replaced by I⁻. The initial I⁻ influx rate is computed by fitting the final 11.5 seconds of the data to an exponential for extrapolation of initial slope, which is normalized for background-subtracted initial fluorescence. All compound plates contain negative controls (DMSO vehicle) and positive controls (5 μM Lumacaftor). Fluorescence is measured using a Tecan Infinite M1000 plate reader equipped with a dual syringe pump (excitation/emission 500/535 nm) [2]
Animal Administration	Rats [1] Male rats (n=3 per dose group) are orally administered Lumacaftor in a vehicle consisting of 0.5% Tween80/0.5% methylcellulose/water at a dose volume of 5 mL/kg. The concentration of Lumacaftor in plasma samples is determined with a liquid chromatography/tandem MS method. Pharmacokinetic parameters are calculated by using WinNonlin Professional Edition software.
Kinase Assay	Screening is carried out using a Beckman Coulter Biomek FX platform. In one set of assays, R1070W-F508-CFTR-HRP (R1070W-HRP)-expressing CFBE41o⁺ cells are incubated with 100 μL medium containing 25 μM test compounds and 0.5 μg/mL Doxycycline for 24 hours at 37°C. In a second set of assays, F508-CFTR-HRP (F508-HRP)-expressing CFBE41o⁺ cells are incubated with 100 μL medium containing 25 μM test compounds, 2 μM Lumacaftor, and 0.5 μg/mL doxycycline for 24 hours at 37°C. All compound plates contain negative controls (DMSO) and positive controls (2 μM Lumacaftor). In both assays, the cells are washed four times with PBS, and HRP activity is assayed by the addition of 50 μL/well of HRP substrate. After shaking for 5 minutes, chemiluminescence is measured using a Tecan Infinite M1000 plate reader equipped with an automated stacker (integration time, 100 milliseconds) [2]
References	[1]. Van Goor F, et al. Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809. <i>Proc Natl Acad Sci U S A</i>. 2011 Nov 15;108(46):18843-8. [2]. Phuan PW, et al. Synergy-based small-molecule screen using a human lung epithelial cell line

	yields $\Delta F508$ -CFTR correctors that augment VX-809 maximal efficacy. Mol Pharmacol. 2014 Jul;86(1):42-51.
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源叶生物