

## Protocol to Prepare AP 20187 (Cat. No. 6297)

### In Brief

#### Resuspension protocol for *in vitro* and *in vivo* experiments

1. Prepare a stock solution of AP 20187 (Cat. No. 6297) in 100% ethanol. We recommend [preparing a 42 mM stock solution](#) for use with the protocol for *in vivo* working solution preparation (below).
2. Tightly seal the vial containing the solution. If necessary, vortex until completely dissolved.

This stock solution can be stored at –20 °C.

*NOTE: Keep the vial of AP 20187 stock solution tightly sealed. Leaving the vial open can result in evaporation of ethanol from the stock solution, which will alter the concentration and may cause precipitation issues when preparing the dosing solution.*

#### Preparation of working solutions for dosing AP 20187 *in vivo*

Prepare the AP 20187 dosing solution within 30 min of administration. To conserve your stock solution, we recommend preparing only the volume required for each experiment. To administer 10 mg/kg in a dose volume of 4 mL/kg, a 1.7 mM (2.5 mg/mL) dosing solution of AP 20187 should be used.

To prepare 1 mL of 1.7 mM dosing solution:

1. Pipette 40 µL of 42 mM AP 20187 stock solution into a vial.
2. Add 100 µL PEG-400 (100%) and vortex.
3. Add 860 µL Tween<sup>®</sup>-80 (2%) in water. Upon addition of Tween<sup>®</sup> 80, a cloudy solution may result, this is transient and clears after gentle agitation or vortex.
4. Administer within 30 min by IP or IV injection.

*NOTE: The 1.7 mM dosing solution is near saturation concentration, slight alterations in preparation may cause precipitation. To prepare a dosing solution at a lower concentration, dilute the stock solution to the appropriate concentration using 100% ethanol and follow steps 1–4. This will maintain the final ethanol concentration of 4%.*

*NOTE: Substitution of Tween<sup>®</sup>-80 with Tween<sup>®</sup>-20 can cause solubility issues and is not recommended.*

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## References

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**Baker *et al*** (2016) [Naturally occurring p16<sup>Ink4a</sup>-positive cells shorten healthy lifespan](#). *Nature* **530** 184 PMID: 26840489.

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