

PolyCYPs incubation conditions - recommendations and tips

Shaking / stirring conditions are important to ensure the best % conversions

CYPs need oxygen so never restrict gas exchange - always incubate the reactions with the gas-permeable seals provided. However, CYPs are deactivated by bubbles so do not over agitate the reactions – stick to the instructions provided with the kit.

Reaction vessel

- Use the square well blocks provided
- Round well blocks and Eppendorf tubes have poor aeration resulting in poor conversion

Alternative vessels

- Suggestions are given on page 3 of the instructions but please ask for advice
- Good conversions have been achieved using 0.5ml in 16mm diameter glass tubes with 2 x 5mm stirrers at a speed of 650 rpm
- Don't use larger stirrers!

Shaking conditions

- Hypha use 150rpm on a shaker with a 5cm orbit for in-house PolyCYPs reactions. Other shaker types may have a different orbit which will impact oxygen transfer.
- Use the [handy calculator](#) link on our website for determining the best rpm to use for your shaker. You will need to know the orbit of your shaker in cm in order to use the calculator.

Calculator Examples e.g. for a 2 cm orbit shaker

R1 (recommended, original rpm)	150	
d1 (recommended, original orbit in cm)	5.00	
Your shaker's orbit/throw - cm (d2)	<input type="text" value="2.00"/>	enter your shakers orbit/throw in cm
Best shaker speed rpm setting (R2)	237	Use a setting of 237 rpm for your 2 cm orbit shaker

This calculator is just a guide. **Please contact us with your shaker model before using and we can give a recommendation for the best settings to use.**

- The MaxQ Mini 4000 benchtop orbital shaker has a throw of 1.9cm. To achieve the same oxygen transfer of an orbital shaker with a throw of 5cm at 150rpm (as used at Hypha), a speed of 243 rpm is required.
- The Eppendorf ThermoMixer has an orbit of 3mm. We recommend a maximum of 400 rpm if you are using this shaker type in order to prevent splashing during the incubations.
- The Heidolph Titramax / Inkubator 1000 is a vibrating shaker and has an orbit of 1.5mm. Nevertheless we recommend a shaking speed of 400rpm.

If you have any questions or need specific advice for your application, email us at support@hyphadiscovery.co.uk