

Brief Protocol for performing Prozomix kREDy-to-go plate assay screens

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1. Blank plate:

- (a) Aliquot 10 mL INT solution (0.25 mg/mL) into a reagent reservoir.
- (b) Using the microplate sealing film cross-hairs as a guide, carefully add 0.10 mL of the INT solution to each well using a multi-channel (or normal) pipette and mix carefully to reconstitute the freeze-dried enzymes. Change tips between additions, to avoid enzyme cross-talk.
- (c) Incubate at ambient temperature in the dark for up to 24 h (though results may be seen as quickly as 15 min).

2. Assay plate:

- (a) Aliquot 10 mL INT solution (0.25 mg/mL) into a reagent reservoir.
- (b) Add 0.05 mL (or 0.05 g if a solid) target alcohol to the INT solution and stir until fully dissolved.
- (c) Using the microplate sealing film cross-hairs as a guide, carefully add 0.10 mL of the INT/alcohol solution to each well using a multi-channel (or normal) pipette and mix carefully to reconstitute the freeze-dried enzymes. Change tips between additions, to avoid enzyme cross-talk.
- (d) Incubate at ambient temperature in the dark for up to 24 h (though results may be seen as quickly as 15 min).

- NOTE:
- (i) only 1 blank plate is required in the case of multiple assay plates
 - (ii) stack the plates together to ensure they all incubate at the same temperature
 - (iii) the plates can be inspected manually by for instance holding above a white sheet of paper, or alternatively, they may be analysed using a microplate reader at 492 nm. If it is elected to read on a microplate reader, best results will be obtained by first mixing the plates on a plate shaker, then pulse centrifuging them, followed by removal of the cross-hair films.
 - (iv) **OPTIONAL** - confirm interesting results by performing another assay plate. This can be useful, as infrequently sporadic alcohol contamination of pipette tips or other plastic s used can lead to false positives (or negatives, if appearing on the blank plate). As kREDy-to-go plates are provided free of charge, it is thus recommended that interesting results be confirmed before other more time consuming/expensive analyses, such as chiral HPLC or chiral GC, be performed.

3. Typical results (cell "A1" is top left, cell "H12" is bottom right):

3 h

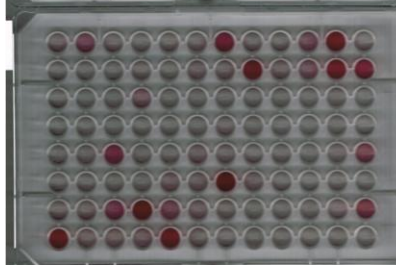
BLANK

1



IPA

2



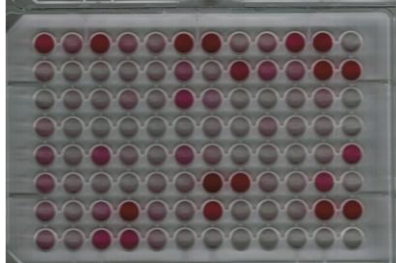
2-Heptanol

3



1-Phenylethanol

4



Ethyl 3-hydroxybutyrate

5

