Application Note – Reductive Amination 96-well plate kit

Introduction

Reductive amination involves the formation of a new carbon-nitrogen bond via the reaction of a carbonyl compound and an amine. This reaction is one of the most widely utilized reactions in discovery chemistry, since the resulting amine products are ubiquitous among biologically active compounds. In a standard reductive amination, the imine/iminium intermediate formed by condensation between an amine and a carbonyl compound is reduced to the amine by the reducing agent. Commonly employed reducing agents for reductive aminations are $NaBH_3CN$ and $NaBH(OAc)_3$. In some cases, the imine/iminium intermediate is preformed prior to the addition of the reducing agent; and in these cases, besides the two hydride reductants mentioned above, the reduction can then also be achieved using $NaBH_4$ or H_2 with Pd/C.

Silica-supported cyanoborohydride was identified as the reducing agent of choice for the Synple automated reductive amination. Combining this solid-supported reductant with a solid-phase extraction strategy, this reductive amination reaction has become more user-friendly, requiring neither manual workup nor time-consuming purification.

Using the approach described in this application note, the Synple Chem 96-well plate reaction kit offers a high-throughtput method to carry out reductive amination reactions between amines and carbonyl compounds.

Kit Composition

- **1x Reaction plate (A)**

96 deep-well plate (2 mL). Contains Si-Cyanoborohydride for the reaction.

(Greiner Masterblock 96-well plate, 2 mL, Polypropylene)

- **1x Purification plate (B)**

96 deep-well filter plate (1.9 mL). Contains SCX-2 for purification.

(Millipore Multiscreen Deep, 96-well Solvinert, Hydrofobic PTFE, 1.9 mL)

- **1x Waste plate (C)**

Empty reusable 96 deep-well collection plate (2 mL) for waste collection.

(Greiner Masterblock 96-well plate, 2 mL, Polypropylene)

- **1x Collection plate (D)**

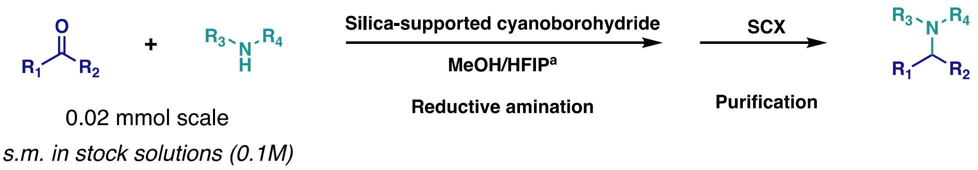
Empty reusable 96 deep-well collection plate (2 mL) for product collection.

(Greiner Masterblock 96-well plate, 2 mL, Polypropylene)

- **Additional required material:**
  - MeOH (HPLC grade) for stock solutions and purification step
  - HFIP (99%+)
  - NH_3 (2.0 M solution in MeOH) for purification step
  - Pipettes or liquid handling robot for transferring liquid
  - Heater+shaker for reaction step
  - Centrifuge or vacuum manifold for purification step

Reaction Scheme

This section describes the general course of the reductive amination with the 96-well plate kit:



a) HFIP = 1,1,1,3,3,3-hexafluoropropanol

References and Publications:

(1) Afanasyev, O. I.; Kuchuk, E.; Usanov, D. L.; Chusov, D. Reductive Amination in the Synthesis of Pharmaceuticals. *Chem. Rev.* **2019**, *119* (23), 11857–11911. Link.

(2) Tripathi, R.; Verma, S.; Pandey, J.; Tiwari, V. Recent Development on Catalytic Reductive Amination and Applications. *Curr. Org. Chem.* **2008**, *12* (13), 1093–1115. Link.

Reaction Planning

Precautions
The Reductive Amination 96-well plate kit is not particularly air and moisture sensitive. The reaction and purification plates contain functionalized silica materials. Be sure the plates are kept horizontally when the plate seal is removed to avoid spilling the solid supported reagents. Be sure the seal is applied tightly during the reaction step and during centrifugation to avoid material leaking.

Solubility
Ideally, the chosen substrates must be soluble in MeOH in order to prepare the initial stock solutions. In case some substrates are not soluble in MeOH, they can be dissolved in alternative solvents like THF or DMF. Please consider that the reaction perfomed with solvents other than MeOH will exhibit a drop in performance (worse conversion and/or purity).

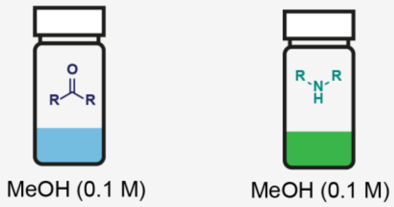
Scale
The Reductive Amination 96-well plate kit is optimized for a scale of 20 μmol (0.02 mmol). On such a scale, in the case of a positive outcome, approximately 2-8 mg of product will generally be obtained. Performing the reaction at higher scale is possible (up to 0.04 mmol), but the performance will drop consistently (worse conversion and/or purity). To perform the reaction at higher scale, is suggested to increase the concentration of the stock solution, rather than increasing the volume of liquids.

Guide for optimal reagent ratios:
To ensure the best outcome from the 96-well plate kit, the reagent ratio shall be adjusted depending on the two reaction partners (for details, see paragraph: *Reaction Procedure – Reaction setup*). In reactions between aldehyde and primary amine, an excess of amine is needed to minimize the double alkylation side product. In reactions with a secondary amine, an excess of carbonyl compound is needed to guarantee an high reagent conversion. If desired, all reactions can be run with 1:1 reagent ratio, but performance drop will be observed (worse conversion and/or purity).

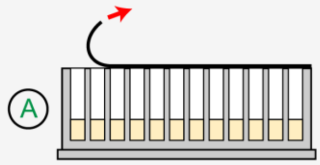
Reaction Procedure

1) Preparation

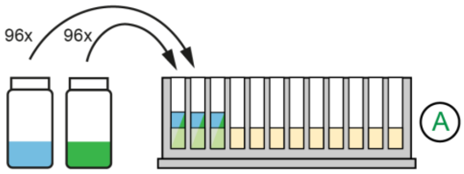
1. Prepare the chosen amine and carbonyl compounds as 0.1 M stock solutions in MeOH.



2. Unpack the **reaction plate A**, being careful to keep it in a horizontal position to avoid spilling the solids contained in the plate, and remove the foil seal.



2) Reaction setup

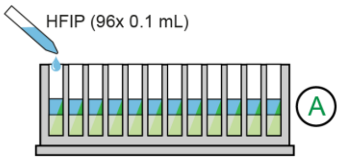


1 With the help of a pipette or a liquid handling robot, add the required amount (see below) of amine and aldehyde/ketone starting materials into the appropriate wells of **reaction plate A**.

Suggested amount of substrate in each well depending on the substrate type (based on 0.1 M stock solutions):

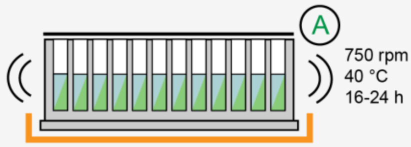
- Reaction between primary amine and aldehyde → 0.2 mL aldehyde + 0.4 mL amine
- Reaction between primary amine and ketone → 0.2 mL ketone + 0.2 mL amine
- Reaction between secondary amine and aldehyde → 0.4 mL aldehyde + 0.2 mL amine
- Reaction between secondary amine and ketone → 0.4 mL ketone + 0.2 mL amine

The ratios suggested above ensure higher yield and purity of the desired product.



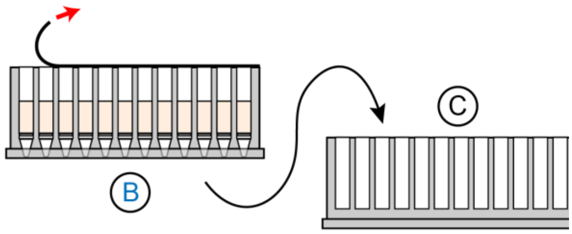
2 When the starting materials are transferred, add 0.1 mL of HFIP to each well of the **reaction plate A**.

3) Reaction



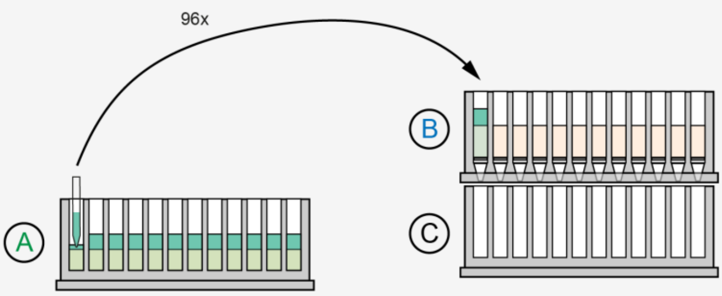
1 Close the **reaction plate A** with the rubber seal. Place the **reaction plate A** onto a heated shaker (or onto a shaker placed inside an oven or incubator) and shake the plate at 750 rpm at 40˚C for 16-24 hours. At the end of this step let the solid settle down for two minutes.

4) Purification

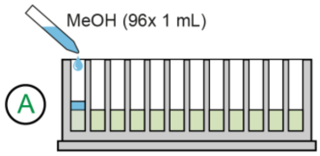


1 Unpack **purification plate B**, being careful to keep it in horizontal position to avoid spilling the solids contained in the plate, remove the adhesive seal and stack the plate over the **waste plate C**.

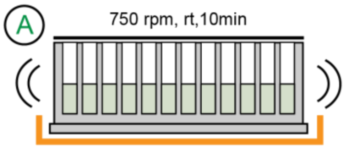
2 Remove the rubber seal from the **reaction plate A**. With the help of a pipette or a liquid handling robot, transfer the crude solutions from **reaction plate A** into the corresponding wells of **purification plate B**, being careful to transfer only the liquid, leaving the solid at the bottom of **reaction plate A**.



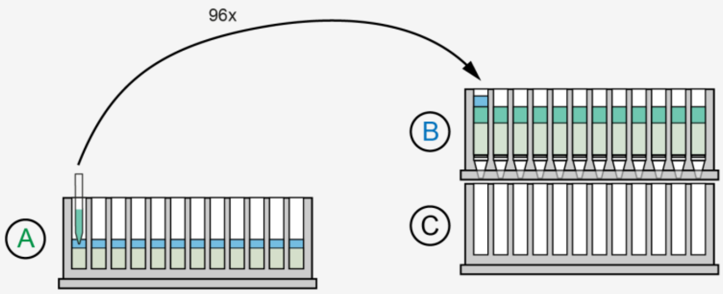
3. After the crude is transferred to **purification plate B**, with the help of a pipette or a liquid handling robot, add 1 mL of MeOH into each well of **reaction plate A**.



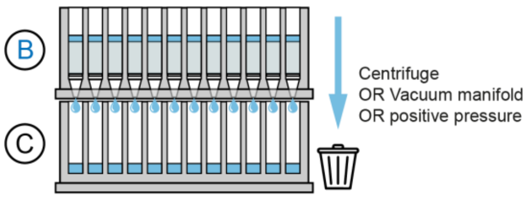
4. Cover **reaction plate A** again with the rubber seal and shake the plate at room temperature for 15 minutes to wash the solid. Afterwards, let the solid settle down for two minutes.



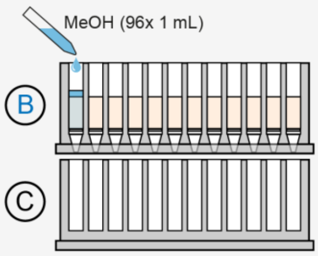
5. Remove the rubber seal from the **reaction plate A**.With the help of a pipette or a liquid handling robot, transfer the liquid from **reaction plate A** into the corresponding well of **purification plate B**, being careful to transfer only the liquid.



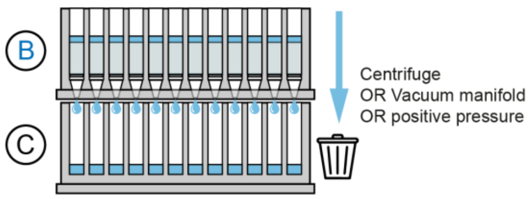
6. Once all the crude material is transferred into **purification plate B**, using a centrifuge or vacuum manifold, filter the crude liquid through **purification plate B** to immobilize the amine product on the solid support. Empty **waste plate C**, which contains waste liquid.



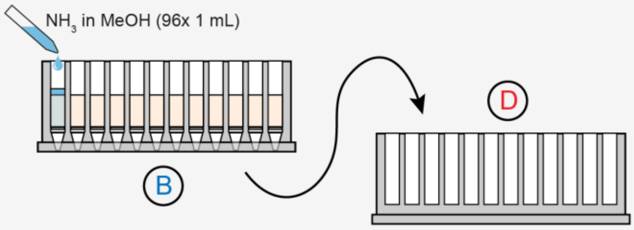
7. With the help of a pipette or a liquid handling robot, add 1 mL of MeOH into each well of **purification plate B**.



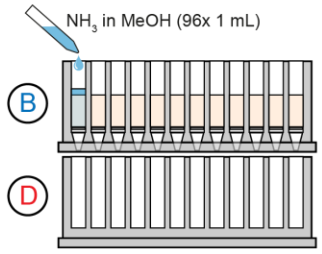
8. Using a centrifuge or vacuum manifold, filter the liquid through **purification plate B** to wash away any non-basic material. Remove **waste plate C** and dispose of the waste liquid.



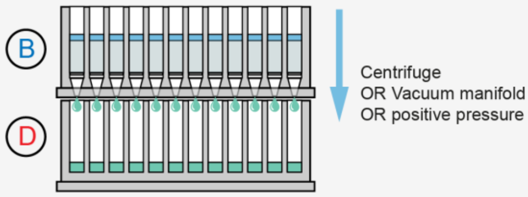
9 Stack **purification plate B** over **collection plate D**.



10 With the help of a pipette or a liquid handling robot, add 1 mL of NH_3 (2 M solution in MeOH) into each well of **purification plate B**.



11 Using a centrifuge or vacuum manifold, filter the liquid through **purification plate B** to release the immobilized product.



12 The liquid contained in **collection plate D** contains the desired product, with any non-basic impurities (e.g. excess carbonyl compound, HFIP etc.) removed.

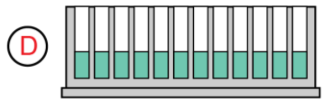


Plate containing product solution

Substrate Scope

Tolerated functional groups

The functional groups tolerated are the same as those tolerated when using the Synple Reductive Amination reaction cartridges with the Synple synthesizer (see Application Note – Reductive Amination https://www.synplechem.com/appnotesdownload)

Example results

To demonstrate the process, a test was conducted on a portion (6x4) of the 96-well plate kit. All the liquid transfer operations were performed in a fully automated way using an Opentrons OT-2 Liquid Handler. Filtrations were performed using a Thermo Scientific Multifuge X4 centrifuge (1000 rpm, 5 min). Purity of the final products was determined by ^{1}HNMR analysis of the crude.

Starting materials used in the experiment:

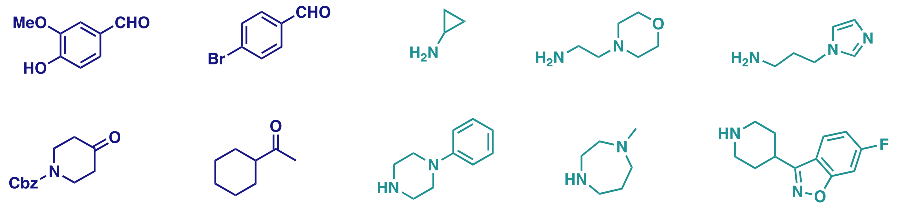
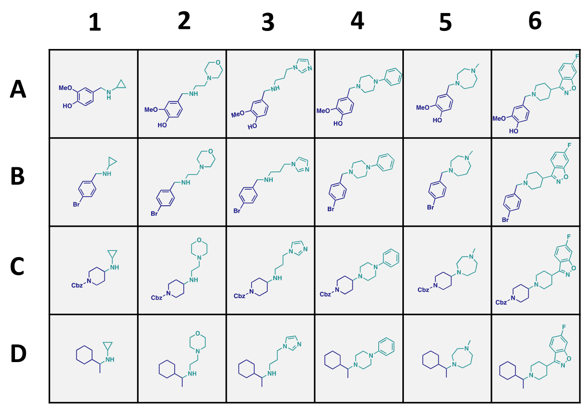


Plate scheme:



Results:

Yield (%)

	1	2	3	4	5	6
A	51	48	57	76	88	70
B	62	70	83	59	81	44
C	73	73	78	66	88	60
D	<5	71	67	0	52	<5

Crude purity (%)

	1	2	3	4	5	6
A	92	38	32	90	95	92
B	85	52	54	67	95	62
C	95	90	90	74	95	66
D	-	95	83	-	90	-

Isolated product (mg)

	1	2	3	4	5	6
A	2.1	2.5	2.9	5.0	4.4	5.4
B	2.8	4.1	4.8	3.2	4.6	3.4
C	4.0	5.3	5.6	5.0	5.8	5.2
D	-	3.4	3.1	-	2.6	-

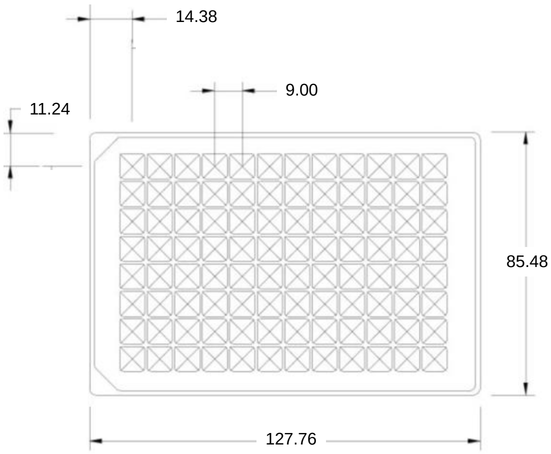
a) Purity determined by ^{1}HNMR of the crude mixture
b) In green: product isolated with ≥90% purity directly after the reaction, no additional purifications performed

This experiment was conducted using both primary and secondary amines, together with aldehydes and ketones, to test the efficacy of the method for all kinds of substrate pairs. In 21 out of 24 cases the desired product was obtained (success rate of 87.5%), with a yield between 44% to 88% and an amount of isolated product between 2.1 – 5.8 mg. In 11 out of the 21 successful reaction, the product was obtained directly after evaporation of the crude solution with a purity of ≥90% without the needs of further purification. The most common impurity found in the crude product was the starting amine, indicating incomplete reaction, while in some cases a small amount of CN addition or double alkylation was observed (see Application Note – Reductive Amination https://www.synplechem.com/appnotesdownload). In the case of wells A2, A3, B2, B3, the low purity is due to the excess amine (2 equiv, see *Reaction Procedure – Reaction setup* section of this document) added to minimize the formation of double alkylation side product. Indeed, less than 5% of such side product was obtained thanks to this precaution.

General parameters

Parameters for Liquid Handling Robot (dimensions in mm)

Reaction plate A:



Purification plate B:

Purification plate B + plate C/D

Equipment for reaction step

The reaction step was performed using a Heidolph Titramax 101 shaker placed inside a Eyela SLI-400 incubator set at 40˚C. Alternatively, a thermoshaker device can be used. Be sure that the rubber seal is correctly applied on top of the plate to avoid any loss of material and to minimize evaporation of the solvent during the reaction.

Equipment for filtration step

Centrifuge: the filtration by centrifugation was tested on a Thermo Scientific Multifuge X4 centrifuge, setting the rotation speed at 1000 rpm and centrifugation time at 5 minutes. Be sure that the rubber seal is correctly applied on top of the plate to avoid any loss of material from the top, and then apply the rubber bands around the two stacked plates to make them adhere as much as possible.
Vacuum manifold: the filtration with vacuum manifold was tested on a Merck Multiscreen® HTS, equipped with deep-well collar. The deep-well collar is necessary to make it compatible with collection plate included in the kit and with the waste reservoir.
Positive pressure manifold: this kit was not tested with positive pressure manifold. Positive pressure was applied manually and no issues in the filtration were observed.