

Improving the efficiency of coating plates and flasks with collagen

Introduction

Coating dishes or tissue culture flasks with extracellular matrix proteins, such as collagen or fibronectin, is a common procedure in cell culture laboratories. However, coating large numbers of dishes is a tedious, time-consuming task that

is repetitive for the user. This application note describes how using the DOSE IT results in significant time savings and reduces excess strain compared to a regular pipette controller.

Key benefits:

- The DOSE IT allows fast and accurate coating of culture flasks or dishes without excessive strain on the user
- CUSTOMIZE mode enables easy and efficient creation of personalized liquid handling protocols
- The DOSE IT has a very small footprint and weighs only 3.5 kg, making it perfect for benchtop or in-hood dispensing applications
- A large display and clear user guidance support fast set-up and use
- The DOSE IT is an intuitive, programmable peristaltic pump that simplifies the dispensing of cell culture media, buffers and other solutions for volumes ranging from 0.1 ml to 2500 ml

Overview: How to coat Petri dishes with collagen

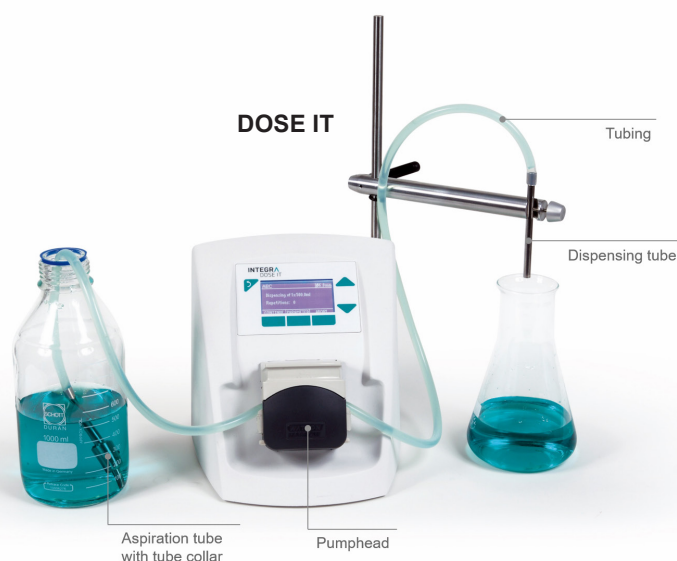
Experimental set-up

All three protocol steps involving liquid transfer were analyzed by measuring the time taken to process 100 Petri dishes, using either a pipette controller in combination with 10 ml serological pipettes or the DOSE IT peristaltic pump.

Before coating the Petri dishes, a collagen stock solution should be prepared according to the manufacturer's instructions. This stock solution should be diluted to a final concentration of 50 µg/ml collagen in 0.02 M acetic acid.

Overview of the steps:

1. DOSE IT set-up
2. Coating the Petri dishes with collagen
3. Removing the excess collagen solution
4. Washing the coated Petri dishes



Step-by-step procedure:

1. DOSE IT set-up

STEP: Tubing adjustment, priming and calibration for dispensing the solution

HOW TO: Open the pump head, adjust the tubing clamps and insert sterilized 4.0 mm tubing into the DOSE IT. Set the flow rate to 470 ml/min (280.7 rpm) for optimal performance. Put the aspiration tube with the tube collar into the collagen solution, and the dispensing tube into an empty bottle to fill the empty tubing with liquid.

Perform the priming and calibration steps according to the [Operating instructions](#).

2. Coating the Petri dishes with collagen

STEP: Dispensing the collagen solution into the Petri dish

HOW TO: Put the dispensing tube above the Petri dish. The DOSE IT directly dispenses the required volume of collagen solution into the Petri dish (**Table 1**). Make sure the entire bottom of the Petri dish is covered with collagen solution. After filling all the Petri dishes, remove the aspiration tube from the collagen solution and put the dispensing tube into an empty bottle. Incubate the Petri dishes at room temperature for one hour.

The DOSE IT can also be used for other culture flasks or dishes, with dispensing volumes given in **Table 1**.

Tip:

- Optimizing collagen concentration and coating times for your cell lines and experimental needs is recommended.

Labware	Recommended coating volume (ml)	Recommended washing volume (ml)
6 well	0.6	1.0
35 mm PD	2.0	1.0
60 mm PD	5.0	2.5
90 mm PD	10.0	5.0
T25	8.0	4.0
T75	24.0	12.0

Table 1: Typical coating and washing volumes.

3. Removing the excess collagen solution

STEP: Aspirating the collagen solution from the Petri dish

HOW TO: After incubation, carefully aspirate the solution from each of the Petri dishes into an empty bottle. The excess collagen solution can be collected in a bottle and reused up to a maximum of 5 times, if desired. Remove the aspiration tube from the used collagen solution bottle, put the dispensing tube into an empty bottle to drain, then drain the collagen solution from the tubing.

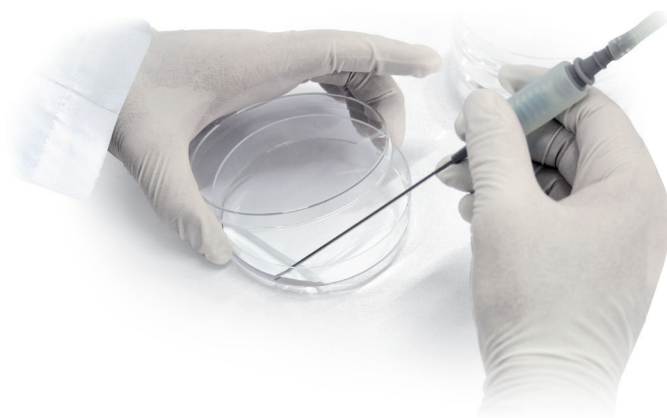


Figure 1: Aspirating collagen solution.

4. Washing the coated Petri dishes

STEP: Rinsing the Petri dishes with PBS buffer to remove the remaining acid

HOW TO: Put the aspiration tube into the PBS buffer (or serum-free medium), and the dispensing tube into an empty bottle to refill the empty tubing. Fill the tubing with PBS buffer, then rinse the Petri dish twice with PBS to remove the remaining acid (see **Table 1** for volumes). Air dry the Petri dishes at room temperature inside a laminar flow hood, then seal with parafilm and store at 4 °C until needed.

Results

For all steps, using the DOSE IT was significantly faster than working with a serological pipette controller, with an overall time saving of 40 % (**Table 2**).

	Pipette controller	DOSE IT	Time saving (%)
Dispense collagen	13 min	6 min	54
Aspirate collagen	15 min	12 min	20
Washing (2x)	60 min	35 min	40
Total	88 min	53 min	40

Table 2: Measured time for handling 100 Petri dishes using a serological pipette controller or a DOSE IT peristaltic pump. Righthand column shows % time saving using a peristaltic pump.

Conclusion

- For all steps, using the DOSE IT was significantly faster than working with a serological pipette controller. When working with a serological pipette controller, all the liquid transfers require a two-step procedure. In contrast, when working with the DOSE IT the same liquid transfer becomes a one-step process.
- In addition to the time savings, the DOSE IT also provides ergonomic benefits. The pipette connector attached to the silicone tubing allows the user to attach any size of aspirator or Pasteur pipette, as required for the individual application.
- The DOSE IT's connector is compact and lightweight, weighing less than 50 grams. This makes it possible to work more ergonomically, and closer to the labware, saving the user from continuously lifting the long serological pipette connected to a much heavier pipette controller. Since the need for repetitive hand movements is eliminated, the risk of repetitive strain injury is minimized.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	171 000	DOSE IT <i>P910</i>	https://www.integra-biosciences.com/global/en/peristaltic-pump/dose-it
INTEGRA Biosciences	171 081	Benchtop switch	https://www.integra-biosciences.com/global/en/peristaltic-pump/dose-it
INTEGRA Biosciences	143 200	Foot switch	https://www.integra-biosciences.com/global/en/peristaltic-pump/dose-it
INTEGRA Biosciences	171 021 171 024	Silicone tubing	https://www.integra-biosciences.com/global/en/peristaltic-pump/dose-it
INTEGRA Biosciences	171 052 171 054	Aspiration/dispensing tubes	https://www.integra-biosciences.com/global/en/peristaltic-pump/dose-it
INTEGRA Biosciences	171 071	Tube collar	https://www.integra-biosciences.com/global/en/peristaltic-pump/dose-it
Greiner Bio-One	633180	Petri dish	https://shop.gbo.com/en/switzerland/products/bioscience/microbiology-bacteriology/dishes/633180.html

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