# Succesful use and handling of FeedPlate® SMFP08002 96-square well, Organism: *E.coli*, Media: Wilms-MOPS Kuhner EquipNote

by Kuhner shaker



### HOW DOES THE FEEDPLATE® WORK?

The FeedPlate<sup>®</sup> is a polymer-based slow release system, which enables fed-batch conditions in small scale bioreactors [Anderlei et al., 2015]. The FeedPlate<sup>®</sup> is applied in biological cultivations in microtiter plates, high-throughput screening, as well as in the process development of shaken bioreactor systems. To start the substrate release, no additional technical equipment is required. After filling the FeedPlate<sup>®</sup> with cultivation broth/media/ liquid, glucose will be released from the polymer. Due to a diffusion driven substrate release, the amount of release substrate depends on applied type of media, pH and osmolality. This application note describes the operation of the FeedPlate<sup>®</sup> in cultivations of *E;Coli* with the synthtic Wilms-MOPS medium. [For recipe see: Appendix]

Note: The FeedPlate<sup>®</sup> is delivered sterile and can be used like a conventional microtiter plate. Please do not autoclave the FeedPlate<sup>®</sup> or reuse it after cultivation. The glucose release in Wilms-MOPS media (pH 7.5) is 7.8 mg/well within 48 h. Other media will show other release kinetics. Therefore, please check your individual substrate release first.

### **APPLYING THE FEEDPLATE®**

Fill the FeedPlate<sup>®</sup> with liquid. A liquid volume of  $250 - 700 \mu$ L per well (96 deep well plate) is recommended. Thus, oxygen transfer rates (OTR) of 2-10 mmol/(L\*h) can be achieved during the fed-batch phase.

For a fed-batch cultivation, the initial glucose concentration in the medium should be reduced. In case of the Wilms-MOPS medium, the glucose stock solution is replaced by DI-water. [see: Appendix]

Note: During fed-batch cultivation, a further secondary substrate limitation (e.g. nitrogen) might occur. For Wilms-MOPS media, ammonia will be limited if 26.7 g/L glucose are metabolized. Please check your cultivation medium for a sufficient ammonia supply.





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### Batch preculture in standard microtiter plate

#### **First Preculture**

Filling volume:	500 µL Wilms-MOPS or complex media with 20 g/L glucose
Shaking conditions:	350 rpm, 50 mm shaking diameter, 37 °C, 80 % humidity
Duration:	between 8 – 24 hours
End biomass conc.:	~ 5 – 10 g/L

#### Second Preculture

Filling volume:	500 μL Wilms-MOPS media with 20 g/L glucose					
Inoculation conc.:	~ 0.1 g/L					
Shaking conditions:	350 rpm, 50 mm shaking diameter, 37 °C, 80 % humidity					
Duration:	8 hours					

OR

### Fed-Batch preculture in FeedPlate®

#### Preculture (2x)

Filling volume:	500 $\mu L$ Wilms-MOPS media with 1-2 g/L glucose
Shaking conditions:	350 rpm, 50 mm shaking diameter, 37 °C, 80 % humidity
Duration:	24 – 30 hours

Evaporation of water should be payed attention in small scale bioreactors such as microtiter plates. High evaporation rates might negatively influence the cultivations results (e.g. increased final product titer). Therefore, cultivation experiments should be run under humidity-controlled conditions (> 80 %). Moreover, the applied sealing foil is essential to reduce evaporation from the wells (see Sieben et al. 2016). With AeraSeal film covers (Sigma-Aldrich), the following evaporation rates per well in a 96-well microtiter plate are known:

37 °C: 3.4 – 3.9 µL/h

30 °C: 2.4 – 2.8 µL/h

Finally, the polymer matrix in the FeedPlate<sup>®</sup> is known to absorb water. For the 96-well FeedPlate<sup>®</sup>, a total amount of 60 µL liquid per well will be lost during cultivation.

### PRECULTURE

Two successive cultivations of precultures are recommended to be performed successively. The best inoculation time for *E.coli* is at highest metabolic activity of the microorganisms. This is reached at exponential or fed-batch growth of the microorganisms. For the first preculture, complex media or Wilms-MOPS can be applied. The second preculture should be run with the same medium which is later applied in the main culture (here Wilms-MOPS medium). If no on-line signal for growth monitoring is available, you should use the following procedures to run precultures (refer to the left side of this page).

Note: If your initial cultures are picked from agar plates and transferred into the FeedPlate<sup>®</sup>, please apply an initial glucose amount of 1–2 g/L glucose in the medium to ensure initial growing of the microorganisms. Wait at least for 24 h before inoculating a second preculture or main culture.





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### Fed-Batch preculture in FeedPlate®

### Mainculture

Filling volume:	250 -700 $\mu L$ Wilms-MOPS media with 0 g/L glucose
Shaking conditions:	350 rpm, 50 mm shaking diameter, 37 °C, 80 % humidity
Duration:	without nitrogen limitation 41 – 115 hours possible

### MAIN CULTURE IN FeedPlate®

Starting ODs in main culture should be between 0.1 - 2 (~ 0.05 - 1 g/L biomass), no additional glucose should be present in the cultivation medium. In Figure 1 the influence of the initial biomass concentration on the dissolved glucose concentration and the initial batch phase during cultivation with FeedPlate<sup>®</sup> is shown. The oxygen transfer rate is a good indicator to distinguish between batch and fed-batch phase [Jeude et al. 2006]. At high initial biomass concentrations (1 g/L) the batch phase occurs over a time period of approximately 2 h. During that period, glucose concentrations stay below 0.5 g/L. In contrast at initial biomass concentrations of 0.05 g/L the batch phase occurs over 10 h and glucose concentrations of 7 g/L are reached.

During batch phase oxygen limitation is possible. If too much glucose is accumulated during the initial growth of the microorganisms, the cultures might get oxygen limited and produce undesired by-products. By using the FeedPlate® oxygen limitation can be avoided. Due to the reduced substrate release at fed-batch conditions, no oxygen limitation will occur if the following shaking conditions are adjusted (refer to the left side of this page).

To avoid oxygen limitations at all, we recommend an inoculum biomass concentration of more than 0.25 g/L and sticking to the procedure of preculturing mentioned above.

Note: Glucose is accumulating during the initial cultivation time in the FeedPlate<sup>®</sup>. At extended microbial lag-phases (e.g. due to delayed harvesting of the preculture), the glucose concentration in the medium might become critical and by-product might be formed. For example, *E.coli* is able to produce acetate which in turn might inhibit the microbial growth.

#### References

- Jeude M, Dittrich B, Niederschulte H, Anderlei T, Knocke C, Klee D, Büchs J. Fed-batch mode in shake flasks by slow-release technique. BiotechnolBioeng, 2006, 95(3):433-45
- Sieben M, Giese H, Grosch J, Kauffmann K, Büchs J Permeability of currently available microtiter plate sealing tapes fail to fulfil the requirements for aerobic microbial cultivation. Biotechnol.J., 2016, 11:1-14
- Anderlei T, Laidlaw D, Bruellhoff K, Selzer S Slow Release Technology for Small-Scale Bioreactor Operations. Gen Bioprocessing, 2015, Mar 15

To avoid critical glucose concentrations:

- Apply high inoculum concentration (best between 0.1 1 g/L) and run precultures in the above-mentioned way
- Use FeedPlate<sup>®</sup> with lower glucose release
- Increase culture volume (up until 700 μL)





### Succesful use and handling of FeedPlate® SMFP08002

96-square well, Organism: E.coli, Media: Wilms-MOPS

### Kuhner EquipNote

by Kuhner shaker

Name column         Maderia         Moderia         Comparison (model)         Comparison (model) <thc< th=""><th>APPENDIX:</th><th>Recipe V</th><th>Vilms-MO</th><th>PS media</th><th></th><th></th><th>0.06</th><th></th><th></th><th></th><th></th><th></th><th></th><th></th></thc<>	APPENDIX:	Recipe V	Vilms-MO	PS media			0.06							
Component:         Molecular         <			Main so	olution			0.06	_	OTR	с <sub>о вм</sub> = 0	).01 g/L			
NH-JASOL         1320         2527         6.98         6.99         0.01         GL           NA_SOL         14218         7.122         5.0         5.00         2.00         <	Component	Molmass [g/mol]	Molarity in medium [mM]	Concentration in medium [g/L]	Weighed portion for <b>947 mL [g]</b>	Volume per 1L medium	0.05		OTR	с <sub>о, вм</sub> = 1	00 g/L		÷	٠
KHPO.       2748       2722       3.0       3.00         No.50.       242.04       142.84       20.200       41.85       41.85         No.50.       209.027       200.00       41.85       41.85       41.85         Accomposition to 800 mLD-water, august privitin have to 94.7 mL       10000 Trace dements       60.00	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	132.02	52.87	6.98	6.98				— Glucose	$C_{ODM} = ($	).01 g/L			
Ns.Soc, Org       1424       1408       20       200         4 Components inc. 900 mLD-wate, adjust pH with NSCH to 73, fill up to 947 mL auticulue, storage 4 R1       947 mL         Glucose Co <sub>0.BM</sub> = 1.00 g/L         Glucose Co <sub>0.BM</sub> = 1.00 g/L         Glucose Co <sub>0.BM</sub> = 1.00 g/L         Component Motions Involve adjust pH with NSCH to 73, fill up to 947 mL         Motions Motions Motions Involve adjust pH with NSCH to 73, fill up to 947 mL         Motions Motions Involve adjust pH with NSCH to 73, fill up to 947 mL         Component Motions Involve adjust pH with NSCH to 73, fill up to 947 mL         Motions Motions Involve adjust pH with NSCH to 73, fill up to 947 mL         Motions Storage without light at 4         Storage without light at 4 *C.         Stor Glucose B (20, 20, 20, 20, 20, 20, 20, 20, 20, 20,	K <sub>2</sub> HPO <sub>4</sub>	174.18	17.22	3.0	3.00	1				0,BM	5			
MORe         20927         2000         4185         4185         947 mL           4 Component         000 ML Provide algost H with NOH Vector algost P with NOH Vector	Na <sub>2</sub> SO <sub>4</sub>	142.04	14.08	2.0	2.00			I —	— Glucose	c = 1	00 a/l			
*** Component: Inc. 900 mL Divaver, adjutt pH with NaOH to 7.5, fill up to 947 mL.         unacted.ver, storage 4 RT         *** Component:       Molarity in Molarity	MOPS	209.27	200.00	41.85	41.85	947 mL				○0,BM -				
3000x Trace elements           Component         Molensy in medium (mM)         Concentration in medium (g/L)         Weighed portion (medium (g/L)         accumulation of glucose           27.55 (x 7/L)         287.45 (x 6/L)         0.0019         0.00054         0.027           (x50, x 8/H)         289.46 (x 6/L)         0.0019         0.00054         0.027           (x50, x 8/H)         289.46 (x 6/L)         0.0019         0.00054         0.027           (x50, x 8/H)         289.40 (x 0.019)         0.0018         0.0018         0.0018         0.0018           (x61, x 8/H)         0.0135         0.0019         0.0028         0.027         accumulation         of glucose           (x61, x 8/H)         0.0135         0.0019         0.0028         0.027         accumulation         of glucose           (x61, x 8/H)         0.0155         0.0138         0.027         accumulation         of glucose           (x61, x 8/H)         0.028         0.039         0.027         accumulation         of glucose           (x62, x 8/H)         10002 Thismit for (10 glu         inthe         inthe         inthe         inthe           (g/moli)         Molenty in medium (mM)         Concentration in medium (g/L)         ior soon Lig         ior	→ Components in autoclave; storage	ca. 900 mL DI ge at RT	-water, adjust pH	with NaOH to 7.5, fil	l up to 947 mL.		년 0.04	-		<u>//</u>				;
Component         Molarity in Ig/moli         Concentration in medium Ig/Li         Weighed portion for 50 mL [g]           7/SQ, x 7H, 0         287 / 45         0.0019         0.00034         0.027           (xS0, x 5H, 0)         287 / 35         0.0019         0.00034         0.027           (xS0, x 5H, 0)         273 / 30         0.00034         0.027           (xS0, x 7H, 0)         273 / 30         0.00034         0.027           (xS0, x 7H, 0)         273 / 30         0.00034         0.027           (xS0, x 7H, 0)         273 / 30         0.00035         0.0019         0.0039           (xS0, x 7H, 0)         270 / 30 / 300         1.670         1.mL           NEDTA 2H, 0         372 / 0.087         0.033 / 0.0198         0.099           (Licrose- Monohydrate         1100 / 20 / 0.015         1.670         4.0mL [g]           1000K Tagenumwite (2 grL Glucose-Monohydrate - 20 grL, pure Glucose)         40 mL           (Licrose- Monohydrate         1000 / 1 medium [g/L]         for 50 mL [g]         40 mL           (1 g/moli)         Molarity in (g/moli)         Concentration in medium [g/L]         Velophed portion for 50 mL [g]         40 mL           (2 grL) Clucose- Monohydrate         20 / 0.01 / 0.1 medium [g/L]         for 50 mL [g]         50 mL     <			1000x Trac	e elements			/L			- Ä				/
2x50x x5H_0       287.45       0.0019       0.00054       0.027         x50x x5H_0       248.66       0.0018       0.00030       0.015         CoCk x5 H_0       277.30       0.1545       0.00198       0.0098         x50x x5H_0       372.24       0.0897       0.03340       1.670         x5teril fittration, storage without light at 41°C.	Component	Molmass [g/mol]	Molarity in medium [mM]	Concentration in medium [g/L]	Weighed portion for <b>50 mL [g]</b>		0 E 0.03	-		/ -				/
CuSQ, x8H_0       249 60       0.0019       0.00048       0.024         MSO, xH_0       287.38       0.0023       0.005       0.0015         CaCL, x8H_0       287.38       0.00024       0.0025       0.00054       0.027         Scal, x8H_0       287.38       0.00024       0.0025       0.00054       0.027         Scal, x8H_0       277.38       0.00034       0.0029       0.00196       0.0029         All 702       0.0135       0.00198       0.0099       1mt       0.00198       0.0099         Steril filtration, storage without light at 4*°C.       Component       Molarly in Concentration in Weighed portion for 500 mL [g]	ZnSO <sub>4</sub> x 7H <sub>2</sub> O	287.45	0.0019	0.00054	0.027					/ l			- I	1
$\frac{MSG_{0,1}H_{0}G}{GeC_{12}K_{14}H_{0}G} \frac{168 99}{20.0018} 0.00030 \frac{0.005}{0.00074} \frac{0.0027}{20.207}$ $\frac{166(12 6H_{0}G_{1}G_{1}K_{2}H_{1}G_{1}G_{1}G_{1}}{1 600 \frac{1}{2} 20.30} \frac{0.01545}{0.04176} \frac{0.04176}{2.088} \frac{2.089}{0.03340} \frac{1.670}{1.670}$ $\frac{1 \text{ medium light at 4 °C}}{1 \text{ Molmass}} \frac{100 \text{ medium light } 1}{0 \text{ medium light } 1} \frac{100 \text{ medium light } 1.670}{1 \text{ medium light } 1} \frac{100 \text{ medium light } 1}{100 \text{ medium light } 1} \frac{100 \text{ medium light } 1}{100 \text{ medium light } 1} \frac{22}{275} \frac{275}{40 \text{ medium light } 1} \frac{100 \text{ medium light } 22}{2} \frac{275}{2} \frac{40 \text{ medium light } 1}{100 \text{ medium light } 1} \frac{22}{105 \text{ medium light } 1} \frac{22}{105 \text{ medium light } 1} \frac{22}{105 \text{ medium light } 1} \frac{22}{100 \text{ medium light } 1} \frac{275}{100 \text{ medium light } 1} \frac{100 \text{ medium light } 1}{100 \text{ medium light } 1} 100 \text{ medium light $	CuSO <sub>4</sub> x 5H <sub>2</sub> O	249.60	0.0019	0.00048	0.024						acc	cumulat	on	1
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	MnSO <sub>4</sub> x H <sub>2</sub> O	168.99	0.0018	0.00030	0.015		<b>~</b>		i		of	alucosa		1
FeCLs 24b4.0       270 30       0.1545       0.04176       2.088       1         CaCls 24b/2       0.00198       0.00198       0.00198       0.00198       0.00198       1       1       Image: CaCls 24b/2       0.00198       0.001       0.0	CoCl <sub>2</sub> x 6H <sub>2</sub> O	237.38	0.0023	0.00054	0.027				i		UI (	giucose		1
Cacle x 2H <sub>2</sub> O         147.02         0.0135         0.00198         0.0999           Na_EDTA x 2H <sub>2</sub> O         372.24         0.0897         0.03340         1.670           * Sterif filtration, storage without light at 4 *C.         255 Glucose (500 g/L pure Glucose)	FeCl <sub>3</sub> x 6H <sub>2</sub> O	270.30	0.1545	0.04176	2.088	1 mL		1	i					1
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	CaCl <sub>2</sub> x 2H <sub>2</sub> O	147.02	0.0135	0.00198	0.099		0 0,02		i i		/		/	
* Steril filtration, storage without light at 4 °C. $ \frac{25x Glucose (500 g/L pure Glucose)}{IMPORTANT: Only used for batch cultivation.} $ Component Molmass Molarity in Medium [g/L] for 500 mL [g] fo	Na <sub>2</sub> EDTA x 2H <sub>2</sub> O	372.24	0.0897	0.03340	1.670				j j				1	
East Glucose (500 g/L pure Glucose) IMPORTANT: Only used for batch cultivation.         Component       Molimass       Molarity in (g/moli       Concentration in medium (g/L)       Weighed portion for 500 mL (g)	→ Steril filtration, st	orage withou	t light at 4 °C.						i i	· · /			/	
Component       Molmass       Molarity in medium [mM]       Concentration in medium [g/L]       Weighed portion for 500 mL [g]         Glucose- Monohydrate       198.17       111.01       22       275       40 mL         * Autoclave (22 g/L Glucose-Monohydrate = 20 g/L pure glucose), storage at RT       40 mL       0       2       4       6       8       10         Molmass       Molarity in medium [g/L]       Concentration in medium [g/L]       Weighed portion for 50 mL [g]       10 mL         Mosco x 7H_2O       246.36       2.03       0.5       5.0       10 mL         MSSO_x X 7H_2O       246.36       2.03       0.5       5.0       10 mL         Molmass       Molarity in medium [mM]       Concentration in medium [g/L]       Weighed portion for 50 mL [g]       10 mL         MSSO_x X 7H_2O       246.36       2.03       0.5       5.0       10 mL         Midmass       Molarity in medium [mM]       Concentration in medium [g/L]       Weighed portion for 10 mL [g]       10 mL         Molmass       Molarity in medium [g/L]       Concentration in for 10 mL [g]       10 mL         Midmass       Molmass       Molarity in medium [g/L]       Concentration in for 10 mL [g]       with 1 g/L and 0.01 g/L inoculum biomass concentration.		25 IMPOR	5x Glucose (500 g TANT: Only used	g/L pure Glucose) d for batch cultivatio	on.		0.01	accum	nulation	Eed-	hatch nhae			
Glucose- Monohydrate       198.17       111.01       22       275       40 mL         * Autoclave (22 g/L Glucose-Monohydrate = 20 g/L pure glucose), storage at RT       40 mL       40 mL         • Autoclave (22 g/L Glucose-Monohydrate = 20 g/L pure glucose), storage at RT       0       2       4       6       8       10         Component       Molarity in medium [mM]       Concentration in medium [g/L]       Weighed portion for 50 mL [g]       0       2       4       6       8       10         MgSO <sub>4</sub> x 7/H <sub>2</sub> O       246.36       2.03       0.5       5.0       10 mL       0       2       4       6       8       10         MgSO <sub>4</sub> x 7/H <sub>2</sub> O       246.36       2.03       0.5       5.0       10 mL       10 m	Component	Molmass [g/mol]	Molarity in medium [mM]	Concentration in medium [g/L]	Weighed portion for <b>500 mL [g]</b>			ofglu	cose			O.BM	- 1.00	9/L/
Autoclave (22 g/L Glucose-Monohydrate = 20 g/L pure glucose), storage at RT   Image: Autoclave (22 g/L Glucose-Monohydrate = 20 g/L pure glucose), storage at RT   Component Molarity in medium [mM] Concentration in medium [g/L] Weighed portion for <b>50 mL [g]</b> MgSO <sub>4</sub> x 7H <sub>2</sub> O 246.36 2.03 0.5 5.0   • Autoclave, storage at RT Iomation in medium [g/L] Iomation in medium [g/L] Iomation in medium [g/L]   Thiamin- fig/molit Molarity in medium [mM] Concentration in medium [g/L] Iomation of oxygen transfer rate (OTR) and Glucose concentration or with 1 g/L and 0.01 g/L inoculum biomass concentration.	Glucose- Monohydrate	198.17	111.01	22	275	40 mL	0		1			-		
100x Magnesiumsulfate (50 g/L)         Component       Molarity in g/Lington       Concentration in medium [g/L]       Weighed portion for 50 mL [g]       O       2       4       6       8       10         Moderation in growing and the concentration in medium [g/L]       Concentration in for 50 mL [g]       Molarity in for 50 mL [g]       Inom       In	→ Autoclave (22 g/l	L Glucose-Mo	onohydrate = 20 g	g/L pure glucose), sto	rage at RT		0	1	1		1	1		1
Component       Molmass [g/mol]       Molarity in medium [mM]       Concentration in medium [g/L]       Weighed portion for 50 mL [g]       Mole approximation in for 50 mL [g]       Weighed portion for 50 mL [g]       Time       Time       Time       In         MgSO <sub>4</sub> x 7H <sub>2</sub> O       246.36       2.03       0.5       5.0       10 mL       Image: Component in the initiation in medium [g/L]       10 mL       Image: Component in medium [g/L]       10 mL       Image: Component in medium [g/L]       Image: Component in medium [g/L]       Weighed portion for 10 mL [g]       Image: Component in medium [g/L]       Weighed portion for 10 mL [g]       Image: Component in medium [g/L]       Weighed portion for 10 mL [g]       Image: Component in medium [g/L]       Weighed portion for 10 mL [g]       Image: Component in medium [g/L]       Weighed portion for 10 mL [g]       Image: Component in medium [g/L]       Weighed portion for 10 mL [g]       Image: Component in medium [g/L]       Image: Component in me			100x Magnesiun	nsulfate (50 g/L)				0	2	4	6	8		10
MgSO <sub>4</sub> x 7H <sub>2</sub> O       246.36       2.03       0.5       5.0         Autoclave, storage at RT         IOOx Thiamin (10 g/L)         Component       Molarity in medium [mM]       Concentration in medium [g/L]       Weighed portion for 10 mL [g]       with 1 g/L and 0.01 g/L inoculum biomass concentration.         Thiamin-       337.27       0.03       0.01       0.1       1 mL	Component	Molmass [g/mol]	Molarity in medium [mM]	Concentration in medium [g/L]	Weighed portion for <b>50 mL [g]</b>							Time	[h]	
Autoclave, storage at RT       10 mL         Interpretent with the storage at RT       1000x Thiamin (10 g/L)         Component       Molarity in medium [mM]       Concentration in medium [g/L]       Weighed portion for 10 mL [g]         Thiamin-       337.27       0.03       0.01       0.1       1 mL	MgSO <sub>4</sub> x 7H <sub>2</sub> O	246.36	2.03	0.5	5.0		Simulatio	n of oxy	gen transfe	er rate (OT	R) and Gluo	cose con	centra	tion of
IOUOx Thiamin (10 g/L)         Component       Molarity in g/Limedium [mM]       Concentration in medium [g/L]       Weighed portion for 10 mL [g]       With 1 g/L and 0.01 g/L inoculum biomass concentration.         Thiamin-       337.27       0.03       0.01       0.1       1 mL	→ Autoclave, storag	ge at RT		1		10 mL								
Component [g/mol]Molarity in medium [mM]Concentration in medium [g/L]Weighed portion for 10 mL [g]Thiamin- Hydrochloride337.270.030.010.1			1000x Thiar	min (10 g/L)		1	with 1 g/l	_ and U.(	JI g/L Inocl	num biom	ass concen	tration.		
Thiamin- 337.27 0.03 0.01 0.1 1mL	Component	Molmass [g/mol]	Molarity in medium [mM]	Concentration in medium [g/L]	Weighed portion for <b>10 mL [g]</b>									
Hydrochloride	Thiamin-	337,27	0.03	0.01	0.1	1.ml								
	Hydrochloride					TWF								



fed-batch cultivation in microtiter plate

