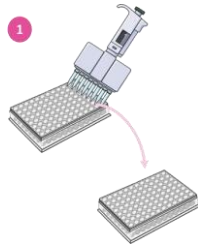


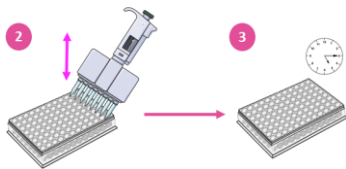
Hydrogel washing step for quantification of molecules

As a hydrophilic natural polymer-based hydrogel, it is likely that BIOMIMESYS® gels may trap some molecules, especially proteins. Prior to the measurement of molecules of interest (protein quantification with ELISA for example) is necessary to perform several washes.

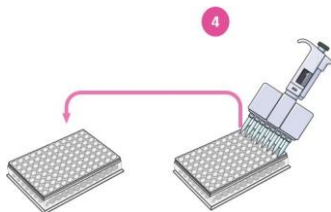
This washing step is described as follows:



- 1) Harvest culture medium (usually 200µL) and keep in separate plate (collecting plate).



- 2) Wash with 250 µL of PBS in cells inside the hydrogel-containing wells.
- 3) Pipet up and down twice and wait 1 minute. Repeat.



- 4) Harvest the 250µL of PBS into the collecting plate.
- 5) Repeat the steps 2, 3 and 4, four times.

- 6) Pool the 4*250µL (=1000µL) of PBS per sample
- 7) Perform dosage as usual or described by the kit manufacturer instructions.
- 8) For the molecule of interest concentration calculation, use the PBS dilution as follows:

$$\text{Dilution factor: } \frac{\text{Total volume of PBS used for washing steps 2-5} + \text{Volume medium step 1}}{\text{Volume medium step 1}}$$



- 9) Example of the mean recovery of known-BSA amounts after 48h of incubation in cell culture conditions with BIOMIMESYS® range hydrogels :

	BIOMIMESYS®	BIOMIMESYS® <i>Hepatocyte</i>	BIOMIMESYS® <i>Adipocyte</i>
Mean	92%	92%	87%
SD	7%	5%	5%