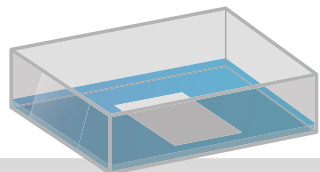


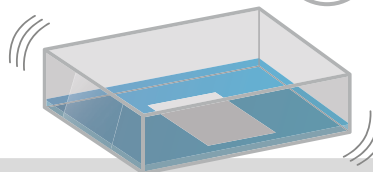
Step **A**

mix 2 mL **A** + 18 mL H<sub>2</sub>O

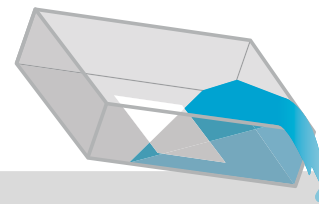


add to membrane

5 min



shake at room temperature

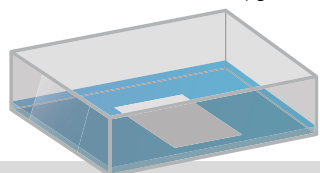


discard Wash Buffer

repeat 3 ×

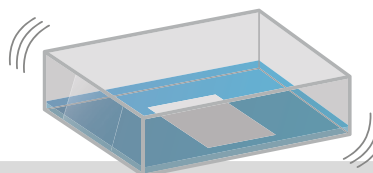
Step **B**

20 mL **B** + primary antibody  
final concentration  
0.2–0.5 µg/mL

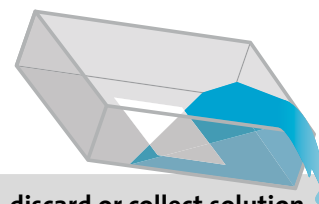


add to membrane

15 min–1 h



shake at room temperature



discard or collect solution  
for further assays

return to  
Step A

repeat Step A (3–4 × 5 min)

start detection