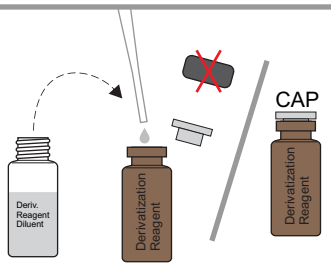
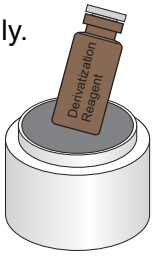
Patulin Sample Prep and Derivatization Flow Chart

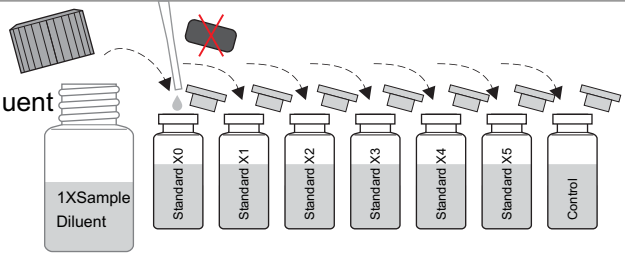
1. Add
Add 0.5mL of Deriv. Reagent Diluent to Derivatization Reagent.



2. Vortex thoroughly.

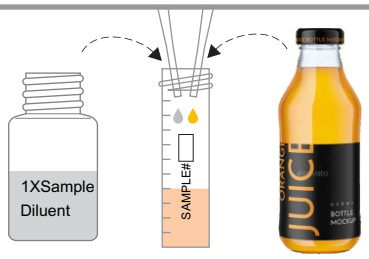


3. Add
Add 1mL of 1X Sample Diluent to each standard to reconstitute.

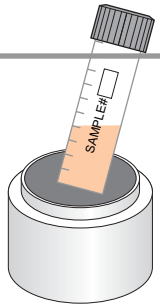


4. Vortex each standard thoroughly.

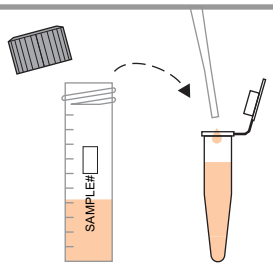
5. Add
For apple/orange juice, add 4.5 mL 1X Sample Diluent to a labeled tube. Then add 0.5mL sample.



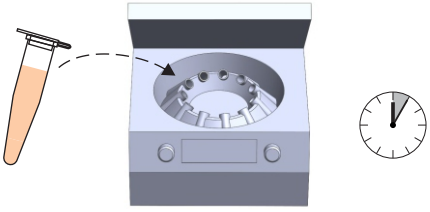
6. Vortex thoroughly.



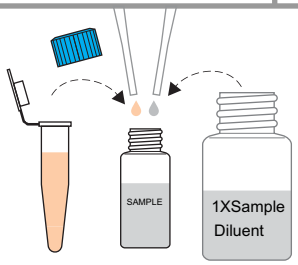
7. Add
Add 2mL of diluted sample to 2mL microcentrifuge tube.



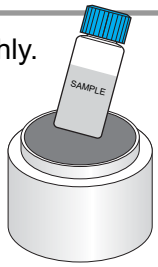
8. Centrifuge
With a balanced centrifuge, spin for 5 minutes at 8000g or 10k rpm.



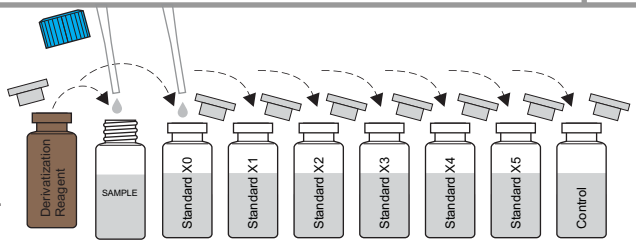
9. Add
Remove 40uL of supernatant and add to 960uL 1X Sample Diluent in a labeled tube.



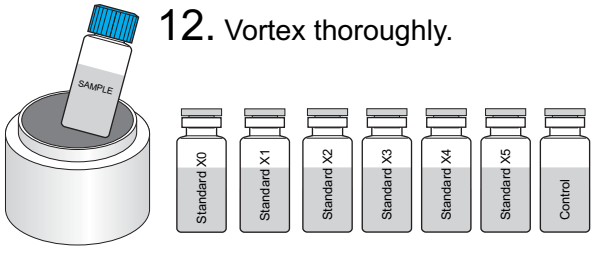
10. Vortex thoroughly.



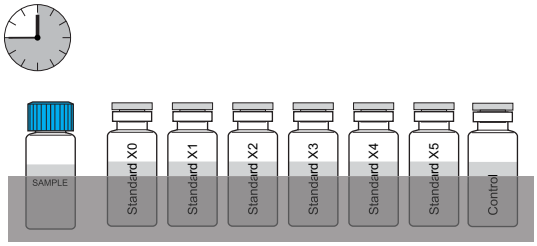
11. Add
Add 10uL Derivatization Reagent to each sample and standard.



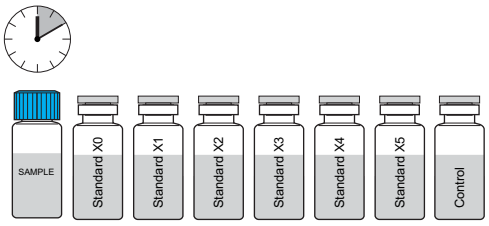
12. Vortex thoroughly.



13. Incubate
Incubate standards and samples at 45°C for 45 minutes in a heat block.



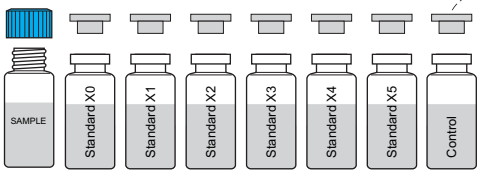
14. Cool
After incubation, let the standards and samples cool for at least 10 minutes at RT.

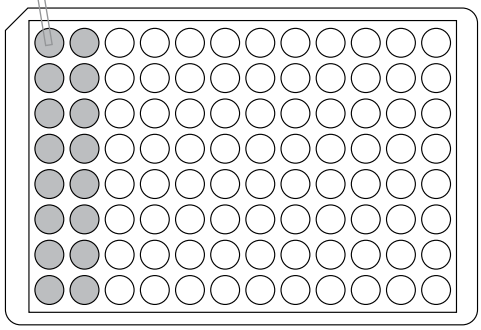


Patulin ELISA Flow Chart

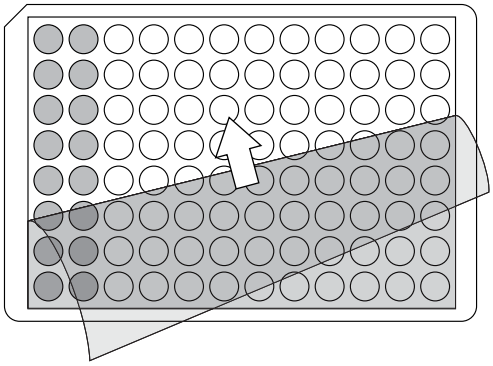
1. Add

Add 100 uL of the **derivatized** standards, control solutions, samples in duplicate into the wells of the test strips according to the working scheme given.



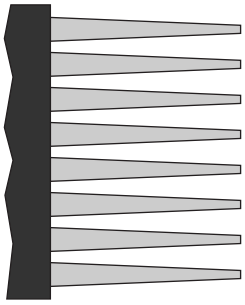


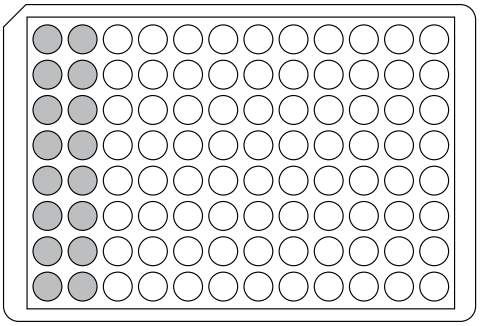
Cover and incubate the strips for 60 min. at room temperature.

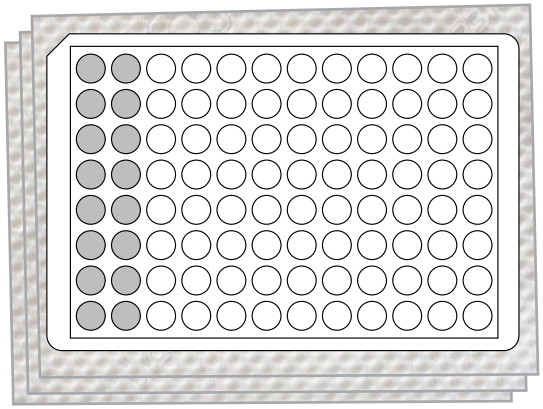


2. Wash

After incubation, remove the covering and discard the contents of the wells into a sink. Wash the strips three times (3x) with a multi-channel pipette or repeater pipette using the diluted 1X washing buffer solution (250 uL of washing buffer for each well and each washing step).



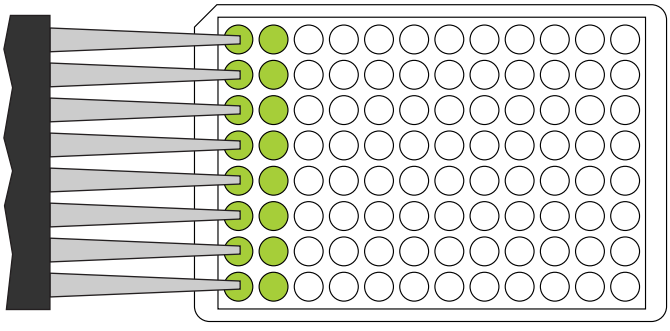




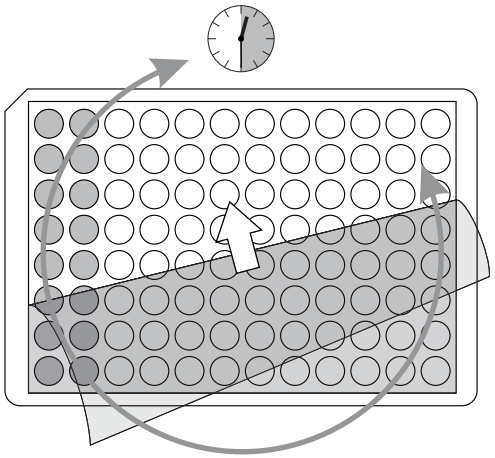
Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.

3. Add

Add 100 uL of the HRP conjugate solution to the individual wells successively using a multi-channel pipette or repeater pipette.



Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop. Be careful not to spill contents. Incubate the strips for 30 min. at room temperature.



Patulin ELISA Flow Chart

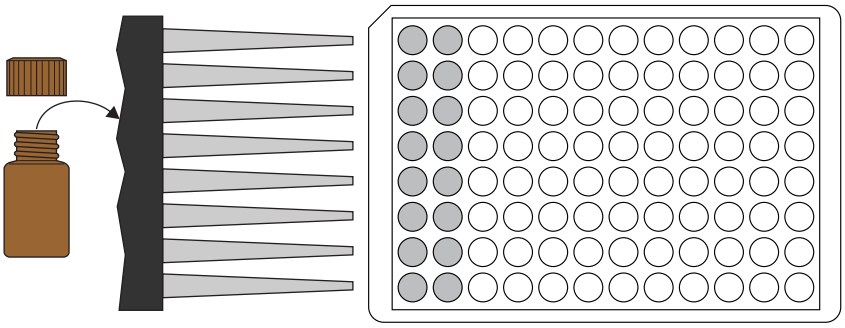
4. Wash (repeat Step 2.)

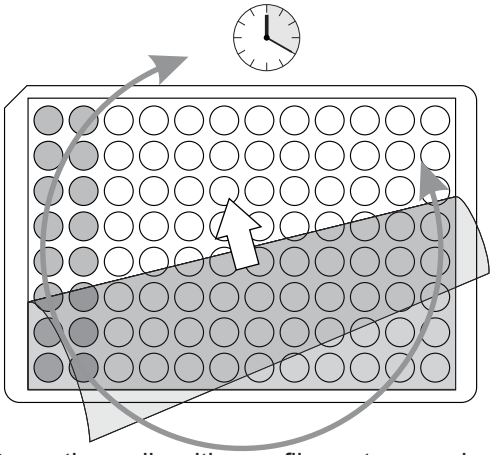
After incubation, remove the covering and discard the contents of the wells into a sink. Wash the strips three times (3x) with a multi-channel pipette or repeater pipette using the diluted 1X washing buffer solution (250 uL of washing buffer for each well and each washing step).

Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.

5. Add

Add 100 uL of substrate/color solution to the individual wells successively using a multi-channel pipette or a repeater pipette.

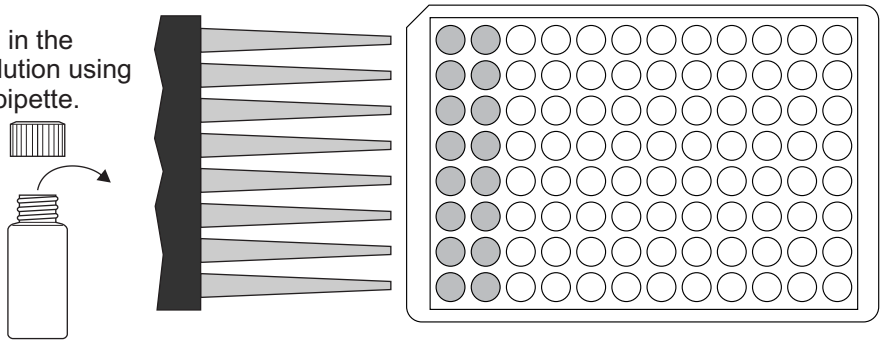




Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop. Be careful not to spill contents. Incubate the strips for 20 min. at room temperature.

6. Add

Add 100 uL of stop solution to the wells in the same sequence as for the substrate solution using a multi- channel pipette or a repeating pipette.



7. Add

Read the absorbance at 450nm using a microplate ELISA reader. Calculate results.