

ELISA in 1 μL on AmpliGrid AG480E

Workflow for ELISA

- Coat AmpliGrid AG480E reaction sites with antibodies against target protein



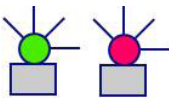
- Incubate with cell culture supernatant containing target protein diluted in buffer (contains BSA and milk powder to block free spaces on AmpliGrid reaction site)



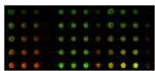
- Incubate with detection antibody against target protein coupled to Biotin



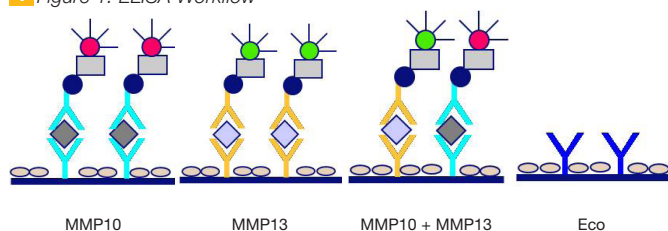
- Incubate with Streptavidin-Cy3 or Streptavidin-Cy5



- Detect Cy3 or Cy5 signals in a fluorescence scanner



1 Figure 1: ELISA Workflow



Detection of MMP10 and MMP13 in chondrocytes

- Pipette 1 μL of antibodies against target protein (anti-MMP10, anti-MMP13, Eco [$c=1$ mg/mL]) in different dilutions on the AmpliGrid AG480F (1:1, 1:2, 1:4 in PBS pH 7.4)
- Incubate over night in a chamber with 100% humidity at room temperature; after that, dry spots at room temperature
- Wash AmpliGrid 3 times 2 min in AdvaWash using washing buffer and centrifuge the AmpliGrid dry using AdvaTube
- Pipette 1 μL cell culture supernatant in different dilutions to the reaction sites and incubate 45 min in a chamber with 100% humidity at room temperature (1:2, 1:5, 1:10, 1:20 in dilution buffer)
- Wash AmpliGrid 3 times 2 min in AdvaWash using washing buffer and centrifuge the AmpliGrid dry using AdvaTube
- Pipette 1 μL of the first biotin labelled detection antibody for the first target protein (Biotin-anti-MMP10) and incubate 30 min in a chamber with 100% humidity at room temperature
- Wash AmpliGrid 3 times 2 min in AdvaWash using washing buffer and centrifuge the AmpliGrid dry using AdvaTube
- Pipette 1 μL of Streptavidin-Cy5 (detection of MMP10) and incubate 30 min in a chamber with 100% humidity at room temperature
- Wash AmpliGrid 3 times 2 min in AdvaWash using washing buffer and centrifuge the AmpliGrid dry using AdvaTube
- Incubate 1 μL of the second biotin labelled detection antibody for the second target protein (Biotin-anti-MMP13) and incubate 30 min in a chamber with 100% humidity at room temperature
- Wash AmpliGrid 3 times 2 min in AdvaWash using washing buffer and centrifuge the AmpliGrid dry using AdvaTube
- Pipette 1 μL of Streptavidin-Cy3 (detection of MMP13) and incubate 30 min in a chamber with 100% humidity at room temperature
- Wash AmpliGrid 3 times 2 min in AdvaWash using washing buffer and centrifuge the AmpliGrid dry using AdvaTube
- Detect fluorescence signals with scanner (e.g., Axon PMT Cy3: 500 nm; PMT Cy5: 700 nm)

Buffers and Materials

Dilution buffer

- 1.5% BSA (bovine serum albumine)
- 2.5% low fat milk powder
- 0.1% Tween20

In PBS pH 7.4

Washing buffer

- 0.1% Tween20

In PBS pH 7.4

AdvaWash with AdvaTube

2 Figure 2: AdvaWash AW400

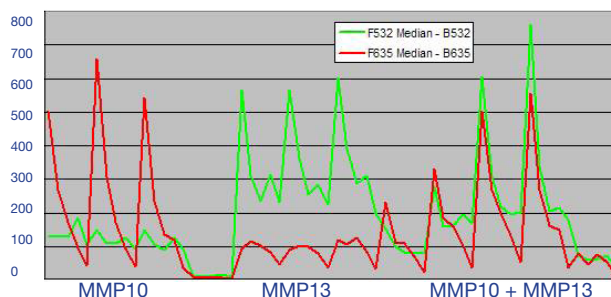


As expected, the target proteins MMP10 and MMP13 could be detected on the AmpliGrid AG480E using ELISA technique.

Figure 3 shows red colored fluorescence signals for detected MMP10 target protein and green colored fluorescence signals for MMP13. The presence of both target proteins MMP10 and MMP13 is visualized by yellow colored signals.

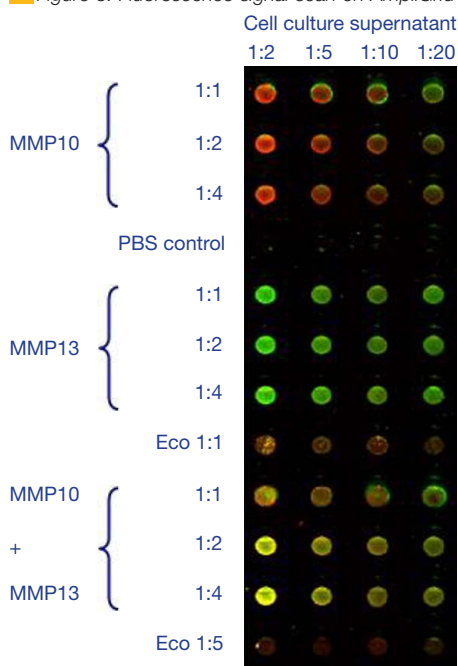
Figure 4 shows the ratio of fluorescence for detected binding to the target protein in comparison to the background fluorescence noise.

4 Figure 4: Fluorescence intensity plot for specific binding detection of target proteins



Results

3 Figure 3: Fluorescence signal scan on AmpliGrid AG480E



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