Immunofluorescent Staining of LMP1 in 721 cells.

The day of staining, 721 cells should be at 2x10^5/ml. NOT more than this concentration.
Leave the plates on the hood for at least 15 min for the cells to sit in the bottom.
Remove most of the media.

1. spin the cells at 1000 rpm for 5 min.
2. take off the media and add 10 mL of acetone:methanol (1:1) for 1x10^8 cells.
3. incubate for 25 min at RT.
4. spin the cells at 1000 rpm for 2min.
5. add 0.1% triton X-100 in PBS. (50ul of 20% triton X-100 and 10mL of 1x PBS).
6. incubate for 10 min at RT with rocking.
7. spin the cells at 1000 rpm for 2 min.
8. remove the 0.1 % triton x-100.
9. add 10 mL of PBS.
10. spin the cells at 1000 rpm for 2 min.
11. repeat step 9 and 10.
12. block the cells with 10 mL of PBS + 5% Calf Serum (2.5mL Serum + 47.5mL PBS)
13. incubate for 30 min.
14. spin de cells at 1000rpm for 2 min.
15. remove the supernatant.
16. add 10 mL of PBS + 5% Calf Serum + 20ul of LMP1 antibody (1:500).
17. incubate for 30 min
18. spin the cells at 1000 rpm for 2 min.
19. remove the supernatant.
20. add 10 ml of PBS + 5% Calf Serum.
21. incubate for 5 minat RT rocking.
22. spin the cells at 1000 rpm for 2 min
23. Repeat step 20-22.
24. remove the supernatant
25. add 10 mL of PBS + 5% Calf Serum + 7 ul of Alexa 488 mouse (1:1500)
26. incubate for 30 min
27. spin the cells at 1000 rpm for 2 min.
28. remove the supernatant.
29. add 10 ml of PBS + 5% Calf Serum.
30. incubate for 5 minat RT rocking.
31. spin the cells at 1000 rpm for 2 min
32. repeat steps 29-31.
33. remove the supernatant
34. add PBS + 5% Calf Serum and leave at 4ºC o/n