Thawing and amplification of iciHHV6 cells immortalized with EBV

1- Thaw a vial of frozen cells in a water bath at 37°C for 2 or 3 minutes.

2- Transfers the cells in a 50mL Falcon tube with 10mL of RPMI 10% FBS

3- Centrifuge 5 minutes at 1200 RPM RT°

4- If there are less than $10X10^6$ cells in the tube put it in a small Flask ($25cm^2$) with 10mL of RPMI 10% FBS keep the flask in an upright position all the time. (Transfers in a medium flask $75cm^2$ once the media turns yellow-this can take up to two weeks).

5- If there are more than $10X10^6$ cells in the tube, put cells in a medium flask ($75cm^2$) with 20mL of RPMI 10% FBS and keep the flask in an upright position all the time.

6- Once in a $75cm^2$ flask, twice a week (usually Monday and Friday), remove ¾ of the culture media without disturbing the cells and add back the same amount of fresh media.

7- **To amplify cells**: Every two weeks, take off ¾ of the media and gently resuspend the cells in the remaining media (5 mL). Take 2.5 mL of cell suspension and transfer into a second 75 cm^2 flask 1containing 17,5 mL of culture media.

8- Do not dilute the cells too much as they prefer to be densely seeded. It is normal that the media becomes orange-yellow and is a good sign that cells are happily growing. If the media remains pink-red after 2 weeks do not split the cells, refresh only the media. Remember to always keep the flask in an up position.