

**Retinal Endothelial Cells – HEC09**

**Figure 1. Expression of CD31 and vWR in Retinal Endothelial Cells.** Cells were stained for CD31 (green) and vWF (red). Overlap of expression of CD31 and vWF is can be seen in the yellow color (expression of both green and red). Each of the 8 wells of the chamber slides were stained (a-h) with both antibodies, to look at the consistency of the cells. There is some variation in the number of cells co-expressing both CD31 and vWF; though within expected range of statistical error.

Protocol: Cells are plated in multi-well chamber slides fixed with 4% paraformaldehyde (10 min at room temperature), rinsed with PBS. Fixed cells were incubated with diluted primary antibodies (CD31 (green, mouse anti-human- BBA7 from R&D diluted to 12 ug/mL) and vWF (red, rabbit anti-human from Abcam ab6994 diluted 1:400 )) for 2 hours at room temperature, rinsed 3 times 15 min each in PBS pH7.4. Then incubated with fluorescent secondary antibodies (anti-mouse Cy2 and anti-rabbit Cy3 from Jackson ImmunoResearch) for 30 minutes at room temp., washed in PBS same as above and mounted using iBright medium (SF40000).

