



Detection of the Beta-2 Adrenergic Receptor (B2AR) in Cultured Human Embryonic Kidney (HEK 293) Cells

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ABSTRACT

HEK 293 is a specific line of human embryonic kidney cells that are derived from a tissue culture. These cells grow relatively fast and, thus, are optimal for use in a research setting. In our experiments, we plated HEK 293 cells, subcultured the necessary amount, and cryopreserved the remaining number of cells. In essence, we cultured cells utilizing an established cell culture protocol. After we cultured the cells, we conducted western blots to determine whether or not the Beta-2 Adrenergic Receptor – the specific protein we were looking for – was present. The Beta-2 Adrenergic Receptor is activated by epinephrine (a neurotransmitter and hormone) receptor found in the plasma membranes of many cell types. After obtaining a sample of human embryonic kidney (HEK 293) cells, we ran the western blots to find that the Beta-2 Adrenergic Receptor was present in the sample of HEK 293 cells that we possessed. As of now, this is what we have accomplished. We can use this information to learn more about the Beta-2 Adrenergic Receptor and the “biased” signaling pathways that many credible sources have found to be true.

BACKGROUND

The Beta-2 Adrenergic Receptor is a member of the adrenergic receptors (adrenoceptors) family. Adrenoceptors are a class of G protein-coupled receptors (GPCRs) – which are proteins that are found in the plasma membranes of cells and transmits a signal to the intracellular molecule, called a G protein – a guanine nucleotide binding protein.

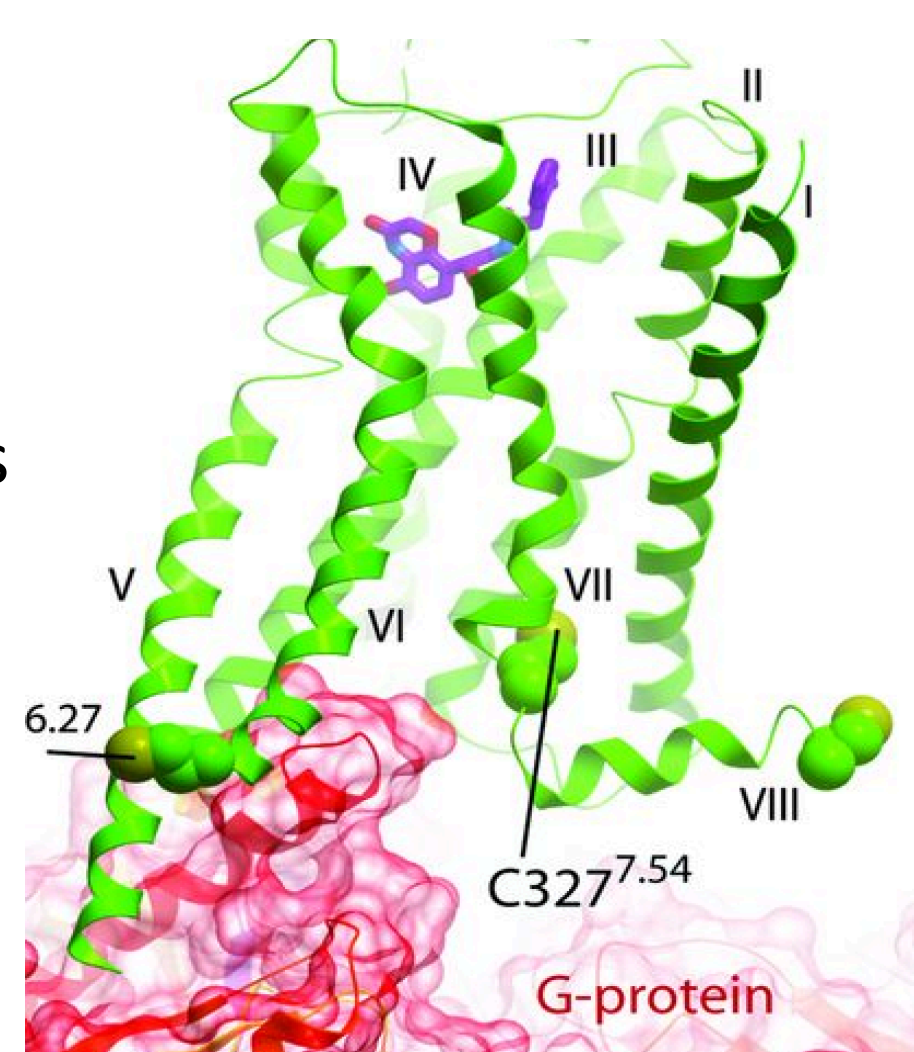
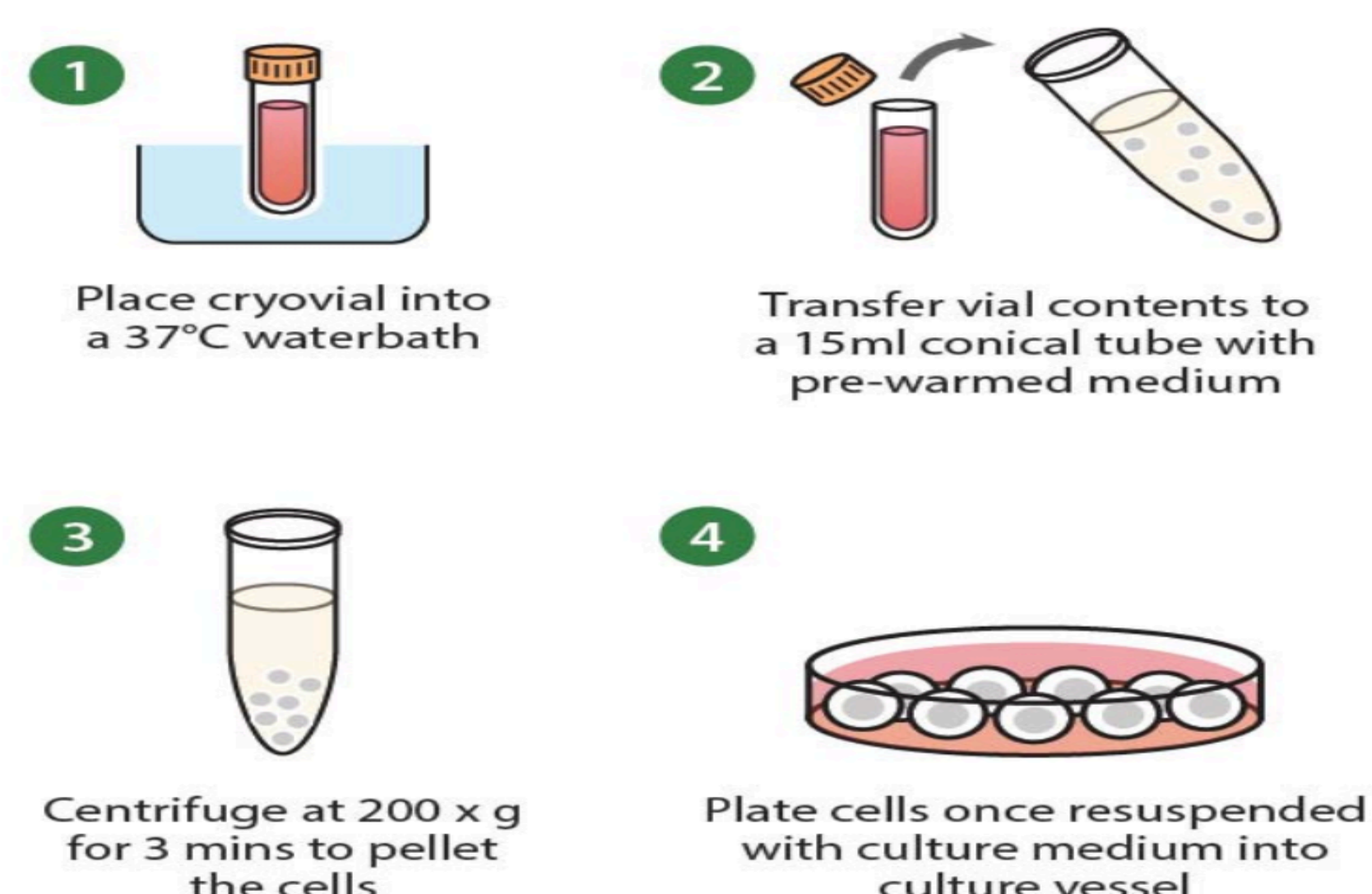


Figure 1: A side view of the activated form of the Beta-2 Adrenergic Receptor

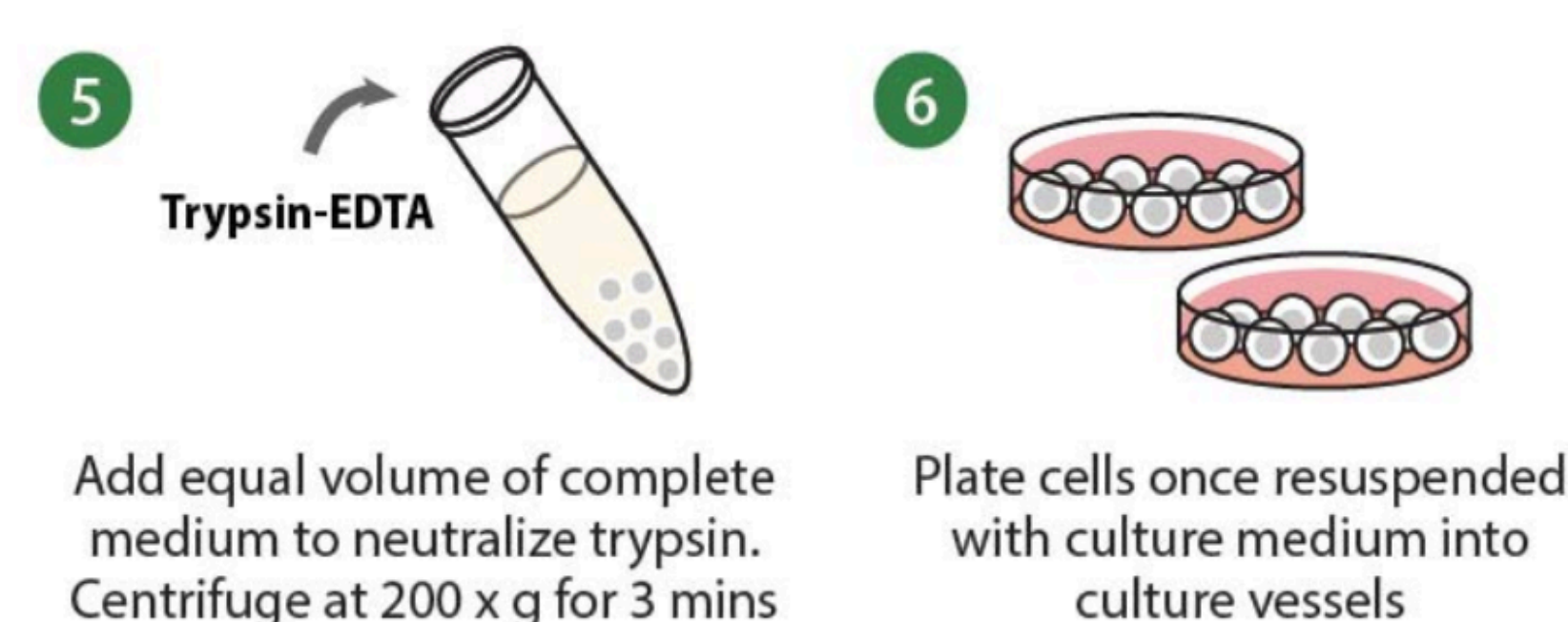
Being one of the most studied receptors in the human body, the Beta-2 Adrenergic Receptor almost serves as a prototype for research purposes. Recent advancements in research have confirmed the conformational changes of the receptor. The Beta-2 Adrenergic Receptor is found in many cells, but in our experiments, we cultured human embryonic kidney (HEK 293) cells. Due to their excellent growth rate and ease of maintenance, human embryonic kidney (HEK 293) cells are a widely used cell type in research. HEK 293 is a particular cell line that originates from human embryonic kidney cells grown in tissue culture.

METHODS

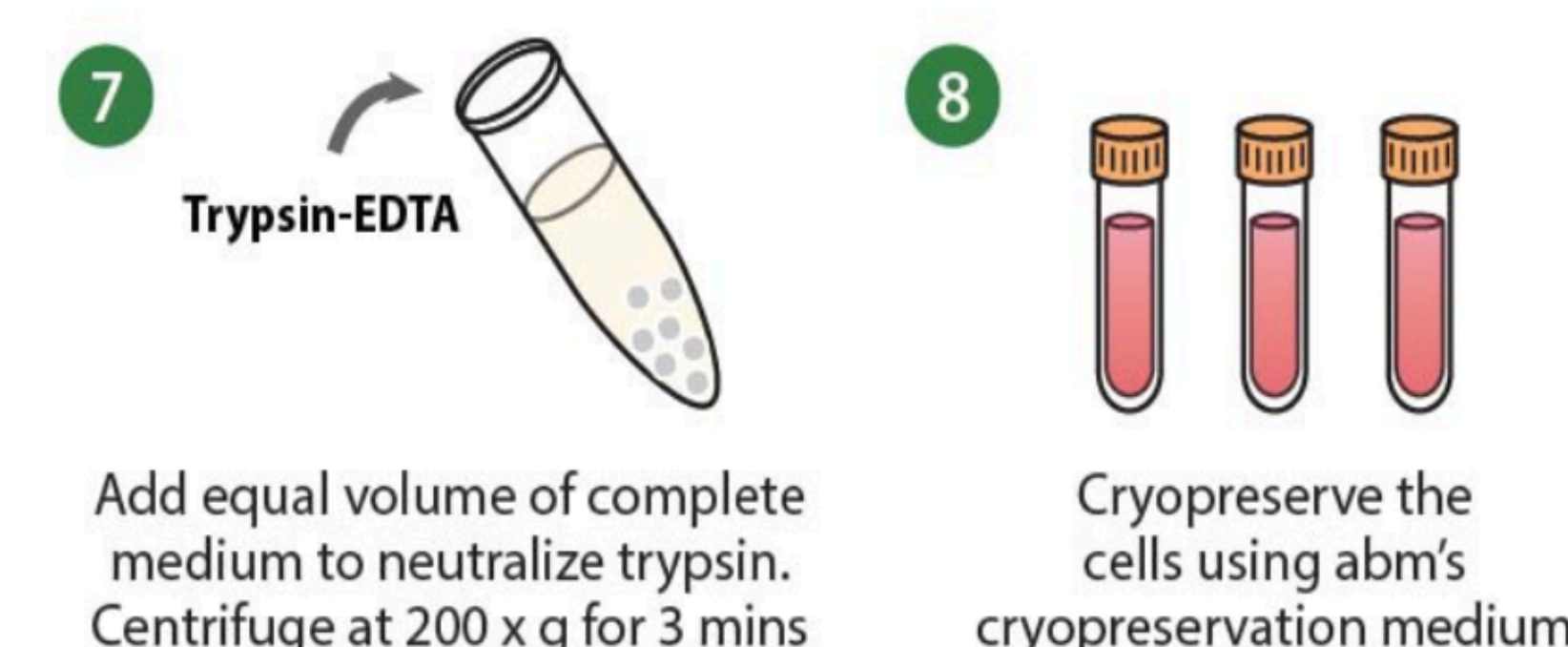
Plating Cells



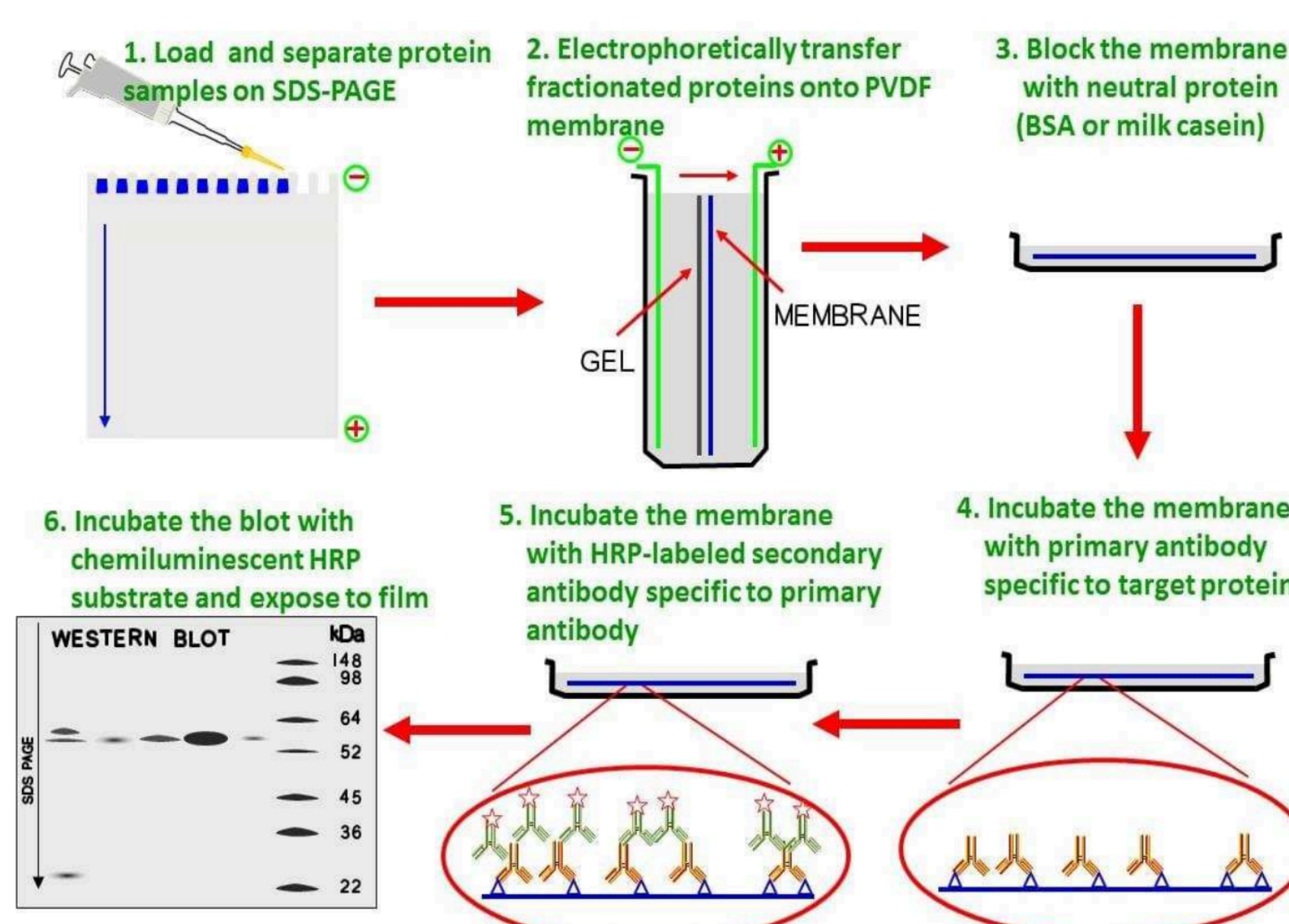
Subculturing



Cryopreservation



Western Blot



RESULTS

Progression of Cultured Human Embryonic Kidney (HEK 293) Cells

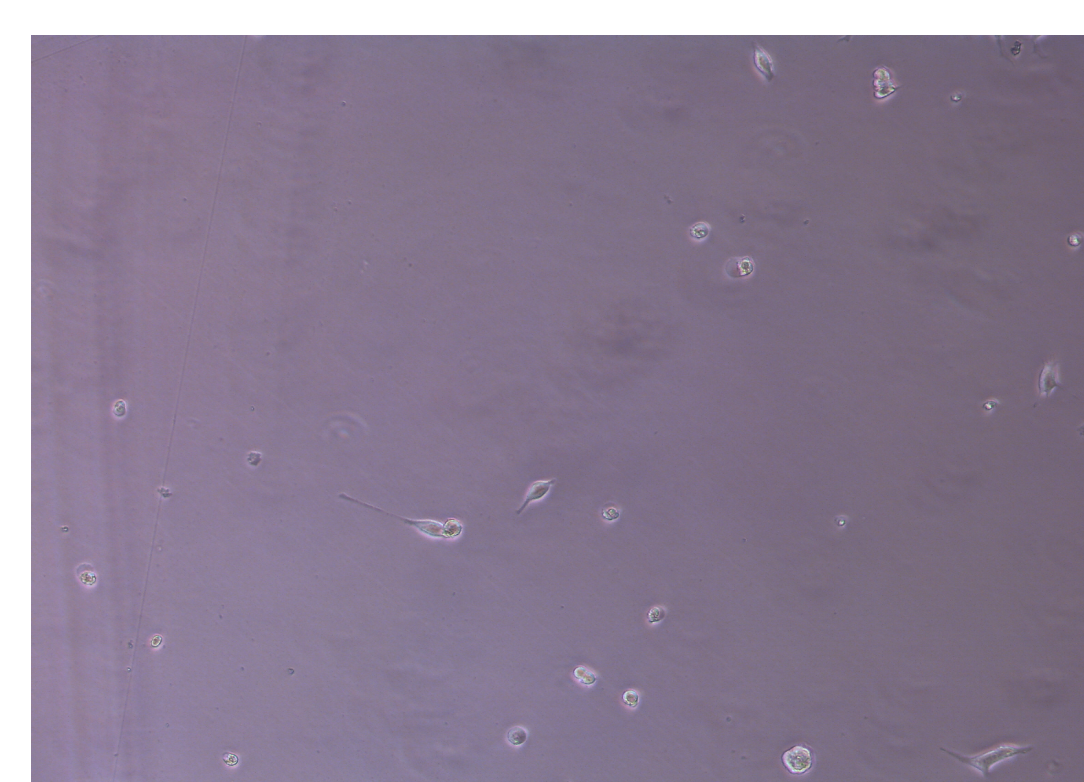


Figure 2: Cells floating in suspension after being plated.

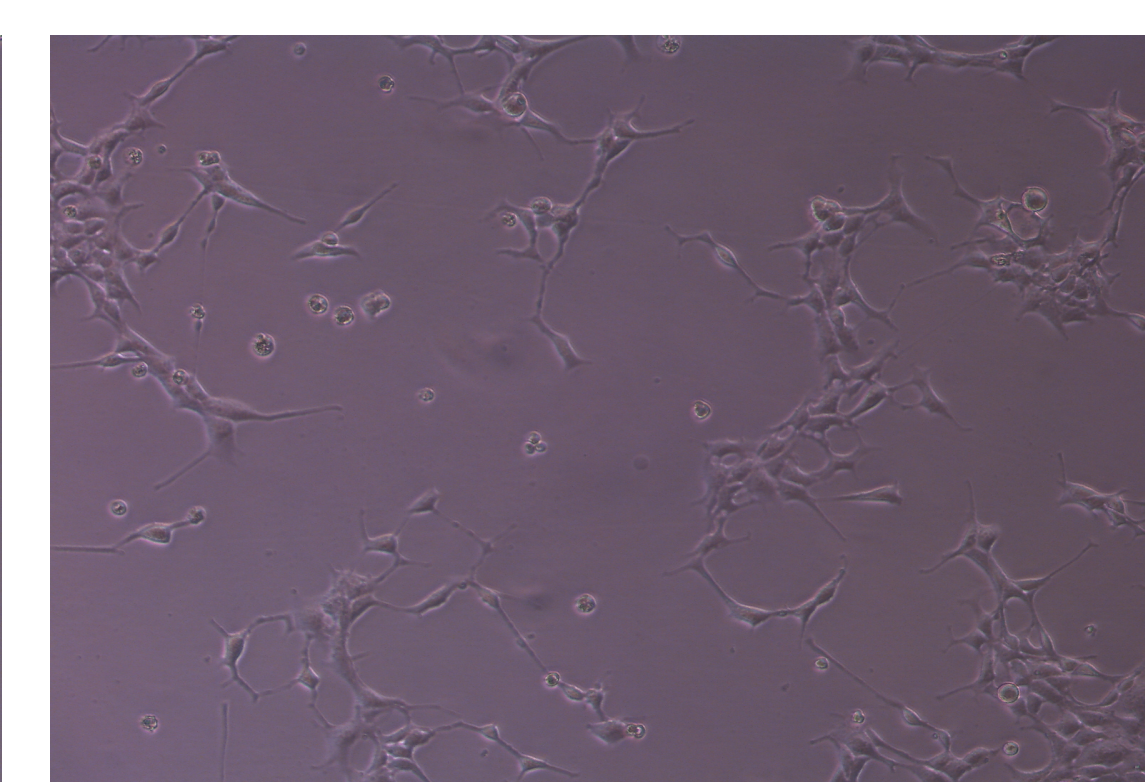


Figure 3: Cells that are attached to the plate that are approximately 20% confluent.

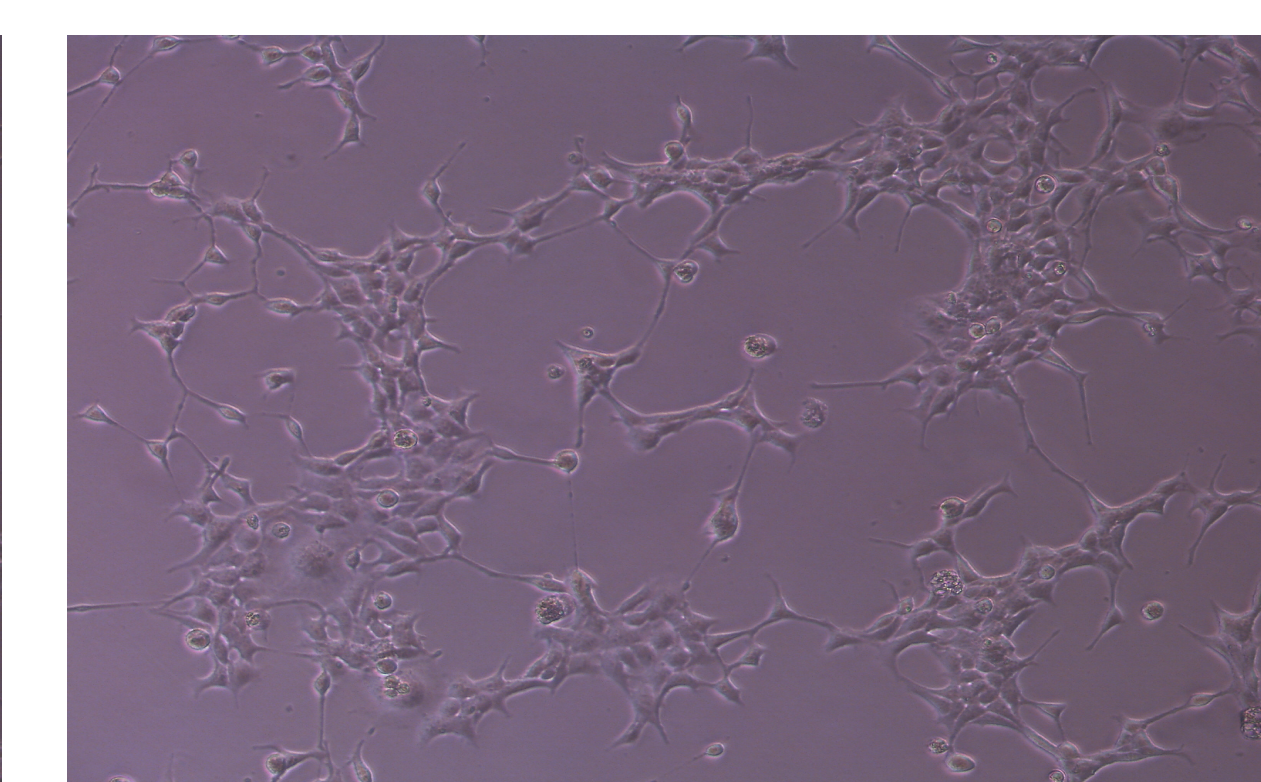


Figure 4: Cells that are attached to the plate that are approximately 30% confluent.

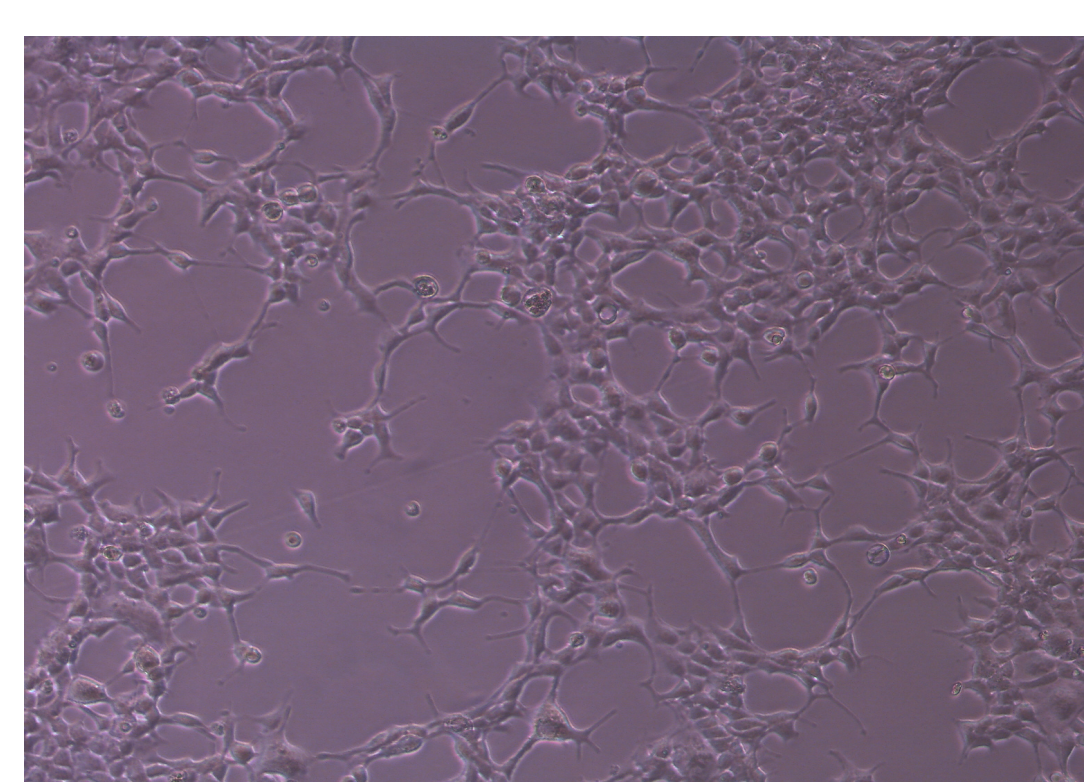


Figure 5: Cells that are attached to the plate that are likely 45-55% confluent. There is evidence of clustered cell growth.

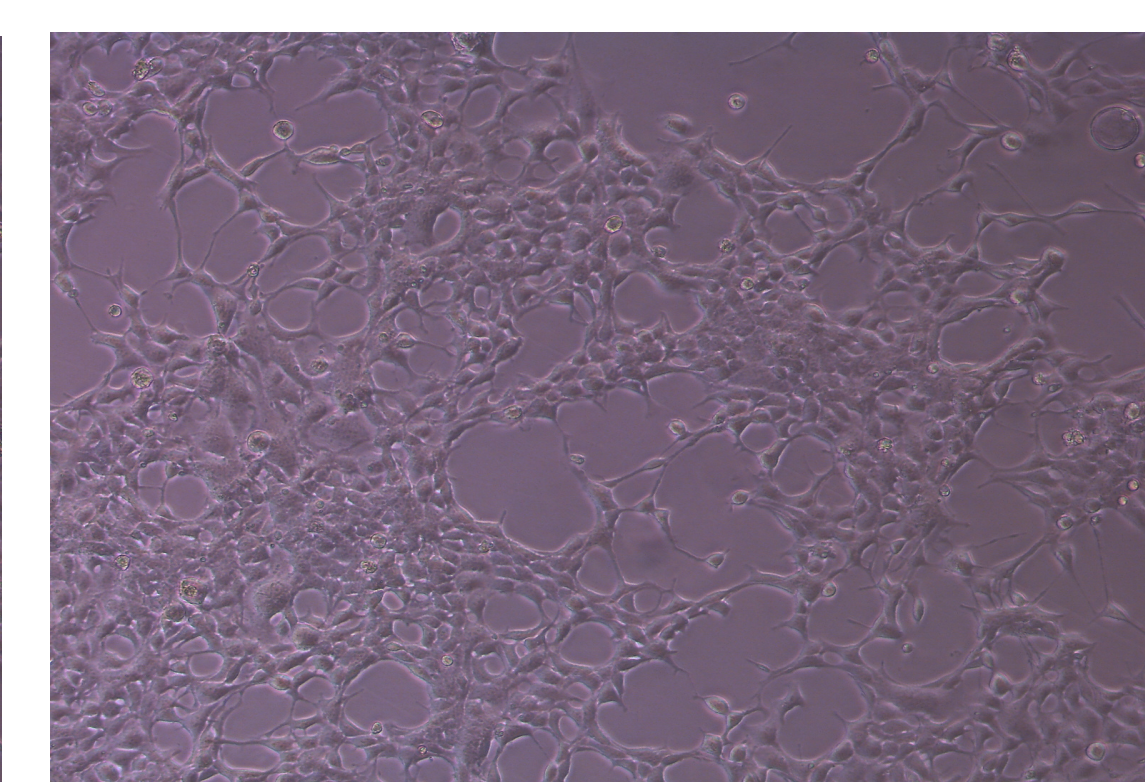


Figure 6: Cells that are attached to the plate that are approximately 60-70% confluent.

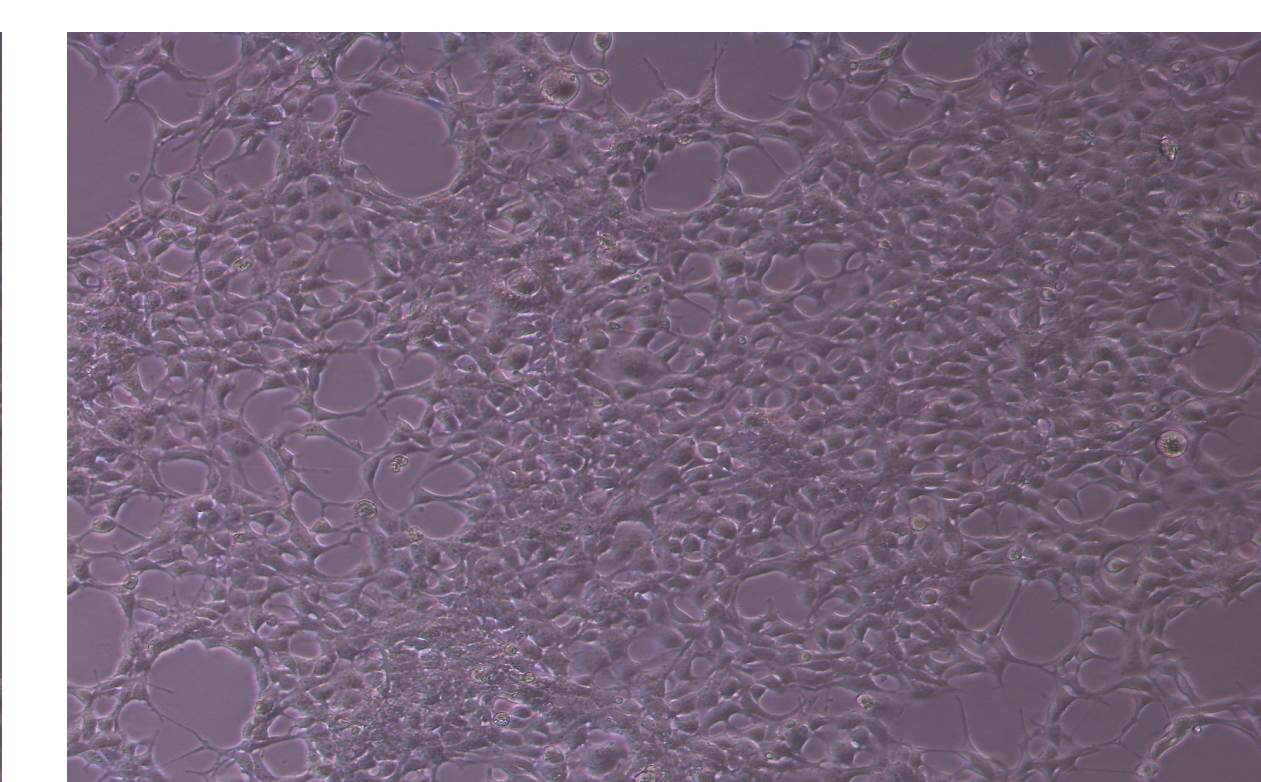


Figure 7: Attached cells that look approximately 95% confluent. They are ready to be subcultured.

Cellular Contamination

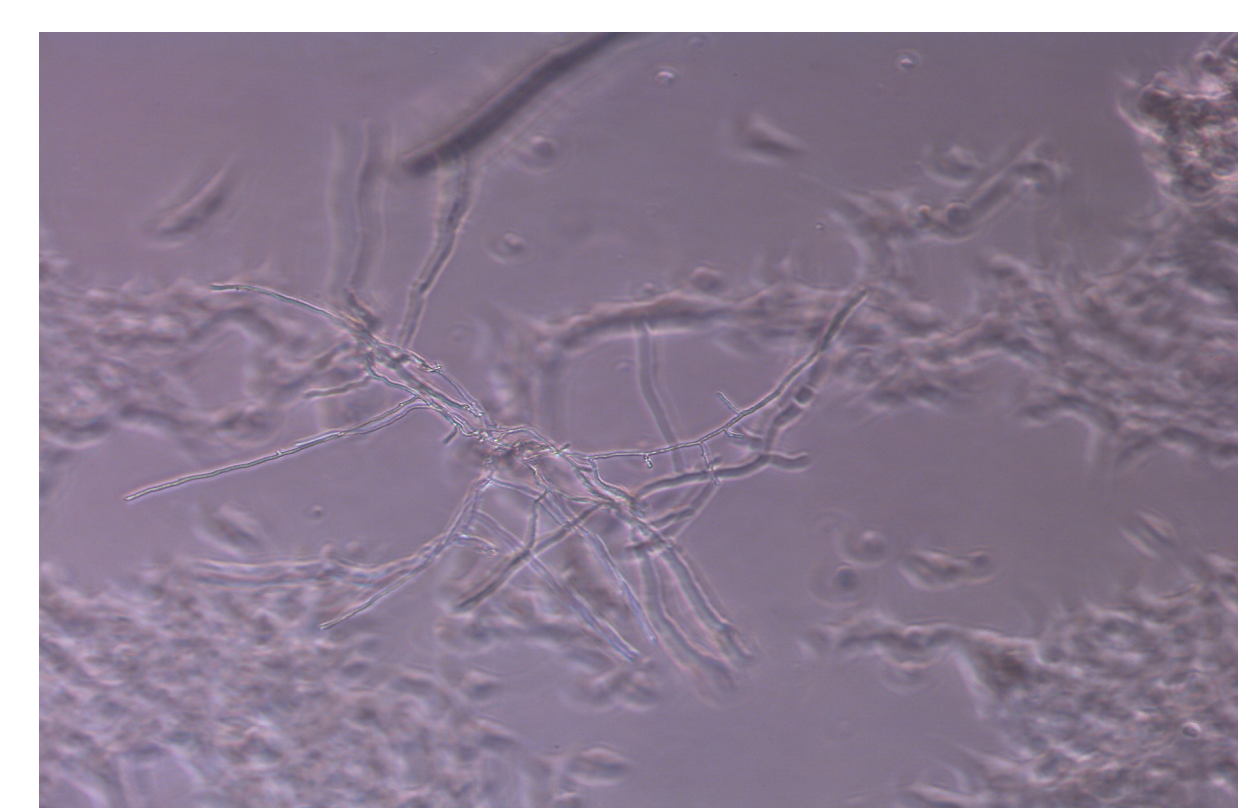


Figure 8: This is an magnified image (100X magnification) of a contaminant suspended in the cell plate. This will lead to fungal contamination in the cell plate and must be treated immediately or discarded. Due to our abundance of cells, we discarded this specific cell plate.

Western Blot

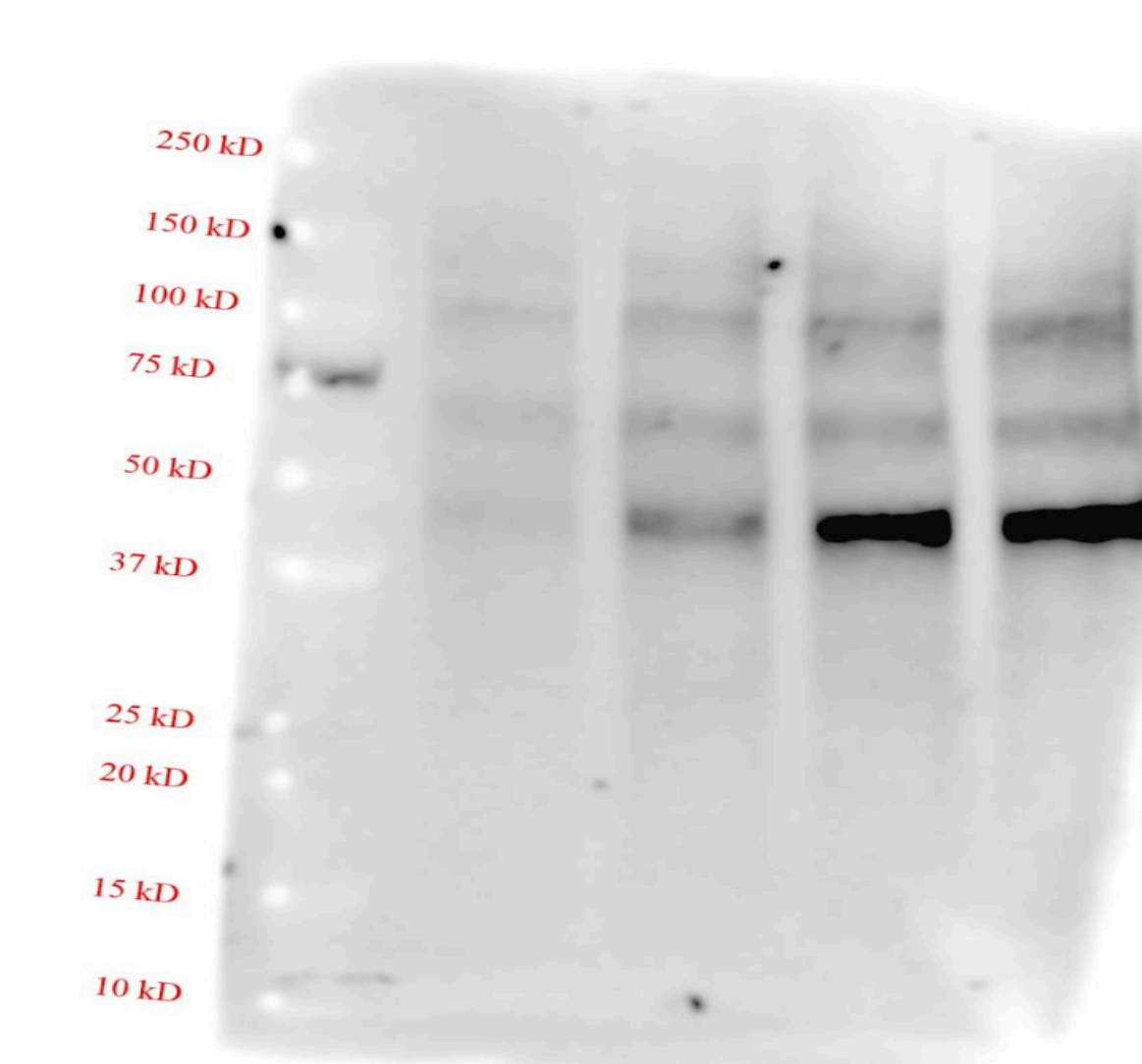


Figure 8: This is an image of the membrane after it has underwent the process of chemoluminescence. The first column is the marker (to detect the molecular weight of the protein in question) and the next four columns are ascending concentrations (15, 30, 45, and 60 µg) of protein. The bands, obtained with the specific antibody for each protein, correspond to the Beta-2 Adrenergic Receptor (B2AR)

CONCLUSIONS

We found that the Beta-2 Adrenergic Receptor (B2AR) was present in the plasma membranes of the samples of human embryonic kidney (HEK 293) cells that we cultured. Now, we can use our information to analyze the B2AR to a further extent. We can focus on the receptor's change in conformation and prepare to transfect the cells with specific drugs that can help us better understand the theory of “biased” cell signaling.

ACKNOWLEDGEMENTS

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