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Detection of Folate Receptor from FR+ Cancer Cells

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Detection of Folate Receptor from FR+ Cancer cells.

A Project

Submitted

to

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By

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of

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Dedicated to

My Family

And

My Advisor

Acknowledgement

I am thankful to my project advisor Dr.Henne, who gave me a chance to work in his research lab and assisted me throughout the project.

I believe that that this project could not be completed without the inspiration of my family and my friends.

It was a good experience to work with Mr.Kuldeep reddy Vanga, my project partner.

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Abstract:

The aim was to detect cancer cells by using folate–PEG-Biotin probe with fluorescently labeled streptavidin for targeted drug delivery of anti-cancer drugs and diagnose cancer cells. Folate-PEG-Biotin (synthesized by Dr. Henne and Mr.Rohan Patel) was purified by HighPeformanceLiquidChromatography (HPLC) analysis and with the help of Liquid Chromatography/Mass Spectrometry (LC/MS) its identity was confirmed. Folate is a basic composition of cell metabolism in both synthesis of DNA and proteins. Growing cancer cells require high level of folic acid. Folate Receptor-α (FR-α) is a membrane bound protein having high affinity for folic acid and serves to transport of folate into cells[1]. Poly ethylene glycol work as spacer and reduce the steric hindrance between peptide and biotin. Biotin is a B-complex vitamin, which is having highest non-covalent affinity to streptavidin. Streptavidin biotin conjugate with folate helps in identifying and detection of cancer cells. We used fluorescently labeled streptavidin and incorporated it with Folate-PEG-Biotin Probe to detect cancer cells[2]. We also studied cell capture by adding fluorescently labeled streptavidin along with 1000-fold excess of folic acid. This work was collaborated with Dr. Tim Gsell by using his high-resolution camera capable of capturing multi-fluorophore fluorescent images. Based on this study, further studies may include incorporation of releasable specific Anti-cancer drug to folate-PEG probe.

Introduction

Folate:

Folate is an essential vitaminneeded for the formation of DNA and Protein synthesis in the human body. Folate helps in the formation of healthy red blood cells. Folate with the combination of methionin and vitamin-B6 can help to reduce the risk of lung cancer by two-thirds. However, Folate deficiency can cause DNA precursor imbalance and promotes chromosome breakage, thus increasing the risk of prostate cancer development.The effect of folate on cancer is complex, Adequate level of folate reduce the risk of esophageal, lung, and ovarian cancer but also assist rapid cell division and cell growth of cancer cells even in people who are already suffering from cancer[3].

Folate receptor:

Significantdevelopments have been madein Anti-cancer technology, though Cancer is not completely curable.Major problems with anti-cancer treatments like chemotherapy and hormontherapy is their poor selectivity for cancer cells and severe toxicity to normal cells. Folate Receptor (FR-α)can helpto overcome this problem[4].

Folate-Receptors are found at high to moderate levels in a kidney, lung, brain, and breast carcinomas. It also found in normal cells but at very low level[5]. The density of FR-α increases as the stage of cancer increases[6]. As such, Folate conjugation to anti-cancer drugs will improve drug selectivity and decrease side effects. Folate conjugation allows a drug molecule to target and become endocytosed into FR- Positive cancer cells[7].

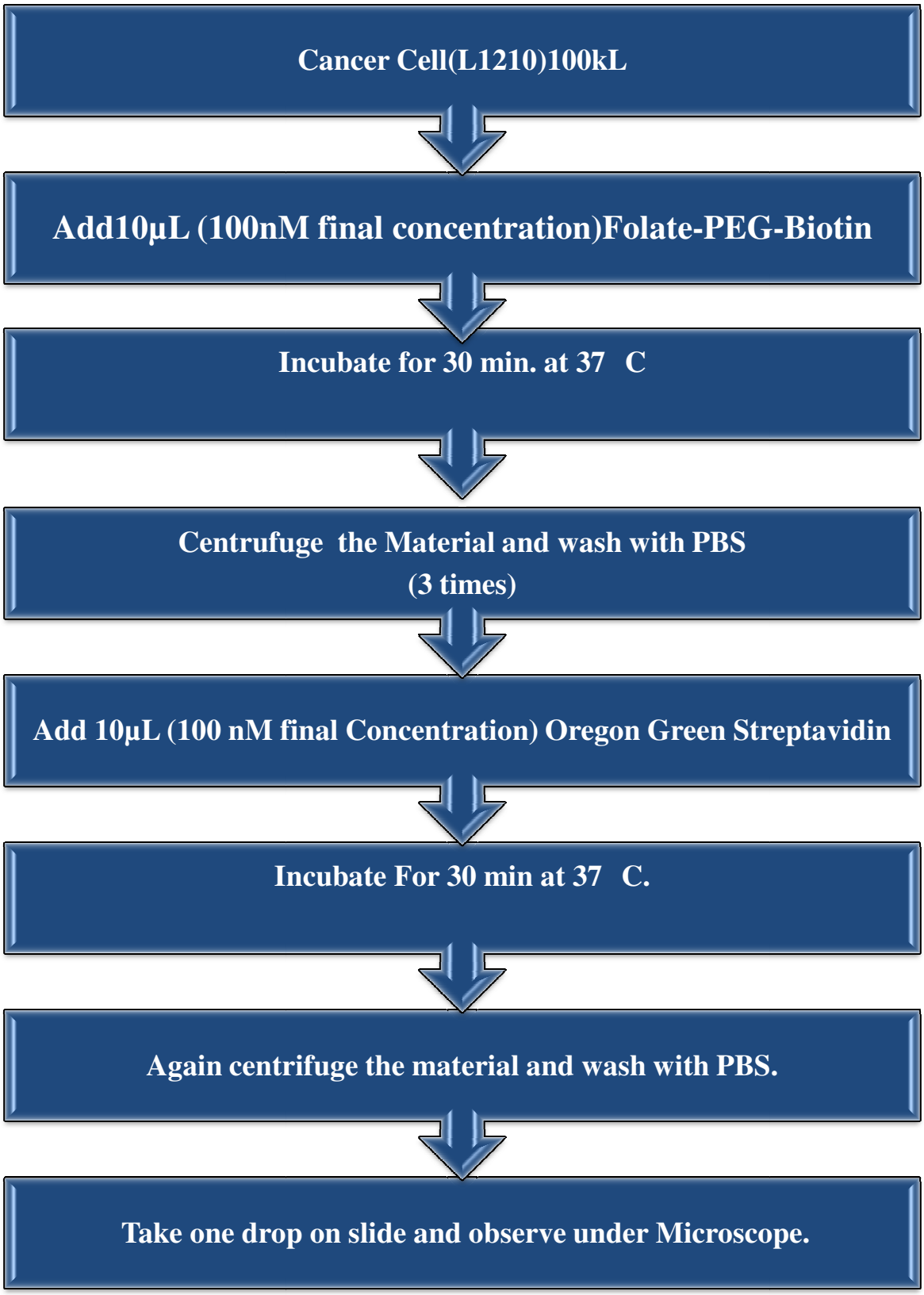
Folate-PEG-Biotin:

We have used Folate-PEG- Biotin with fluorescently labeled streptavidin for detection and diagnosis ofcancer cells. Folate-PEG-Biotinis inexpensive and can be more easily produced than other more costly anti-body based conjugates. Biotin is a B complex vitamin, which canbe chemically linked to proteins like streptavidin. Biotin shows high affinity and specificity for streptavidin[8]. Polyethylene Glycol used as a spacer, increases conjugate solubility and helps to reduce steric hindrance between the cell receptor and streptavidin. Alternative spacer lengths enable optimization of conjugate function for specific biotin-binding assays involving streptavidin. We candetect cancer cells by binding withfluorescently labeledstreptavidin to folate through biotin and observing under fluorescence microscope with a high-resolution camera capable of capturing multi-flouorophore images.

Streptavidin:

Streptavidin is a tetramer protein that has an extremely high affinity for biotin. We have used fluorescently labeled streptavidin labeled with Oregon-green dye. The high affinity of streptavidin for biotin has made it useful for many bioanalytical applications involving the immobilization of proteins, vesicles, and other biomolecules, as well as imaging.

CellStudy


Cancer Cell(L1210)100kL
Add10µL (100nM final concentration)Folate-PEG-Biotin
Incubate for 30 min. at 37 C
Centrufuge the Material and wash with PBS (3 times)
Add 10µL (100 nM final Concentration) Oregon Green Streptavidin
Incubate For 30 min at 37 C.
Again centrifuge the material and wash with PBS.
Take one drop on slide and observe under Microscope.

Materials

RPMI medium 1640

(GIBCO)

Lot # 27016

[+] L- Glutamine

[+] Phenol red

[-] Folic acid.

[Thermo-Scientific]

Product# 22832

Lot# LA142162

1ml(1mg/ml)

PBS 1X

With out calcium and magnesium

Cat. # 21-040-cv

Lot # 21040174

EXP 07/11

Mediatech, INC Cellgro.

Purification and Analysis of Folate-PEG-Biotin

We needed to purify Folate-PEG-Biotin probe (prepared by Dr. Henne and Mr.Rohan Patel) by HPLC method using the Hewelett Packard, series-1050 instrument that is equipped with a Diode Array Detector.

Rigel 5µm C-18 10 X 250 nm columns were used for purification purpose and Ammonium bicarbonate was used as a buffer.

Parameters for **HPLC**:

Solvent A: Ammonium bicarbonate

Solvent B: Acetonitrile

Flow Rate: 1ml/min

Run Time: 60 min.

Column: Rigel 5µm C-18

No	Time (Min)	% Solvent B
1	0.0	1.0
2	5.0	1.0
3	35.0	30
4	45.0	50
5	55.0	60
6	60.0	1.0

LC/MS:

Once we had collected the purified Folate-PEG probe by HPLC method, we needed to further analyze it with LCMS to confirm the identity and check the purity.

We have used eclipse XDB C-18 column with 1% Formic acid in Acetonitrile and water as mobile phase in positive ion mode and eclipse XDB C-18 column with Methanol and water as mobile phase in negative ion mode.

Parameters For **LC/MS:**

Solvent for Positive Ion mode: 1% Formic acid, Acetonitrile, water.

Solvent for Negative Ion mode: Methanol and water.

Flow rate: 0.5 ml/min

Run time: 10 min.

Scale range: 600-1000 m/z

Sample size: 30 µl (200 ppm)

No.	Time in Min	% Methanol
1	0	30
2	1	50
3	2	70
4	3	90
5	4	90
6	5	90

Result and Discussion

We have used HPLC for separation and purification of Folate-PEG-Biotin. By using the reverse phase chromatography technique, we got the separation peak for Folate-PEG-Biotin at around 37 min. The compound, which separated at 37 min, was found to be Folate-PEG-Biotin by DAD spectra, which shows absorbance at 284nm and 350nm. Small peaks indicate some impurities in the compound. Once we purified the Folate-PEG-Biotin, it was further analyzed by LC/MS to confirm its identity. In negative ion mode we got peak at 868 (m-1). The calculated value for Folate-PEG-Biotin is 869. Some fragments occurred at 890 and 891 and may be attributed to the impurities in the solvent.

We did two different cell studies. One was with Folate-PEG-Biotin and Oregon green dye and another with Folate-PEG-Biotin,Oregon green dye, and excess of Folic acid. For the first study, we observed fluorescentlylabeled cancer cells under a fluorescent microscope and these images were captured with a camera. For the second study, the excessof folic acid wastaken by folate receptor, thus preventing the uptake of fluorescently labeled streptavidin. Taken, together these results indicate we have selective binding of the folate probe to the L-1210 cancer cells.

Conclusion:

By using reverse phase chromatography in both HPLC and LC/MS we can, purify and confirm the detection of Folate PEG Biotin respectively. In HPLC, the peak at 37 min and the DAD spectra at 284nm and 350 nm confirm the identity of Folate PEG Biotin. In LC/MS, (negative ion mode) using methanol and water as mobile phase the molecular ion peak at 868(M-1) is that of folate-PEG-Biotin which relates to the calculated molecular weight of that compound. In cell studies fluorescently labeled streptavidin bound to Folate-PEG-Biotin detects and diagnoses cancer cells, however, with an 1000 fold excess of folic acid, the excess of folate binds with the folate receptor and thus cancer cells are not detected.

Funding acknowledgement

This project was funded by Governors State University. Dr.Henne donated HPLC-1050 and LC/MS was made available from GSU. Dr.Henne generously donated several other pieces of equipment supplies.

List of Figures:

Figure1: Folate Uptake pathway.

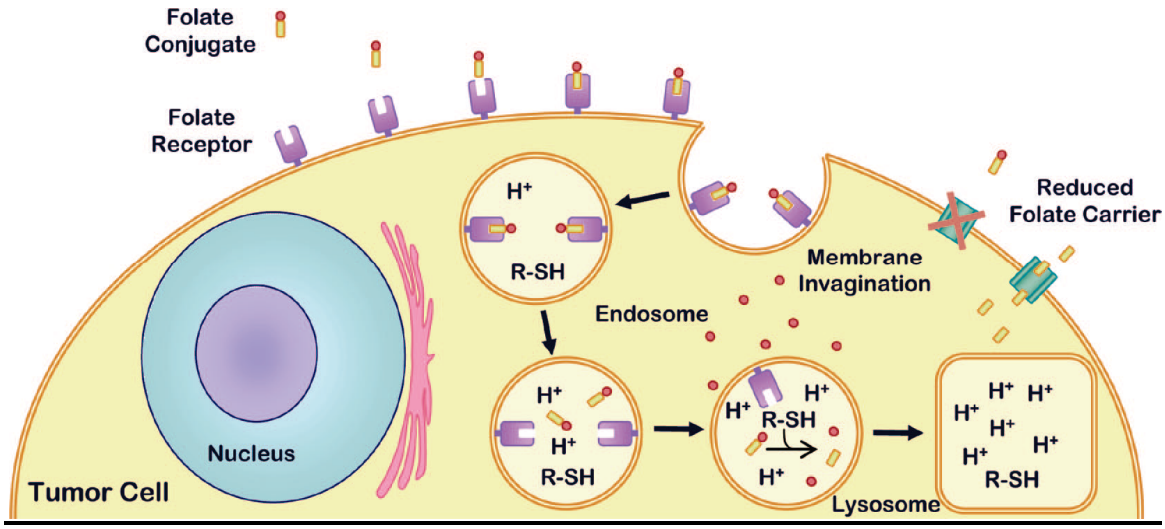


Figure 2: HPLC chromatogram of Folate PEG biotin

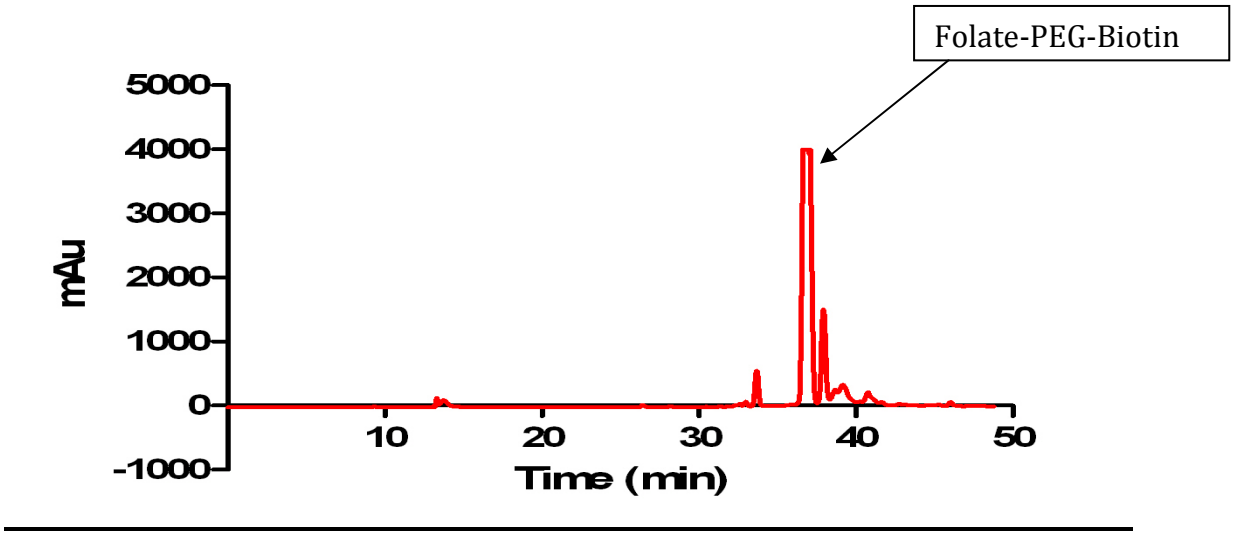


Figure 3: DAD spectra for Folate PEG biotin

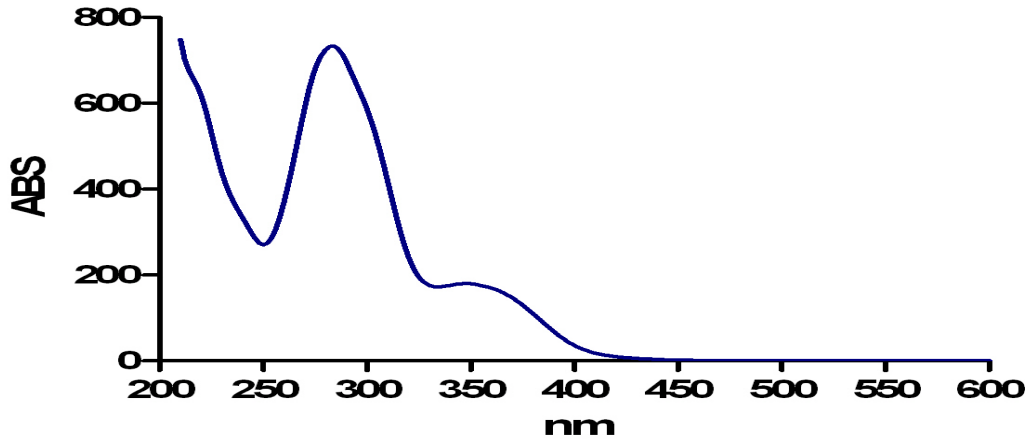
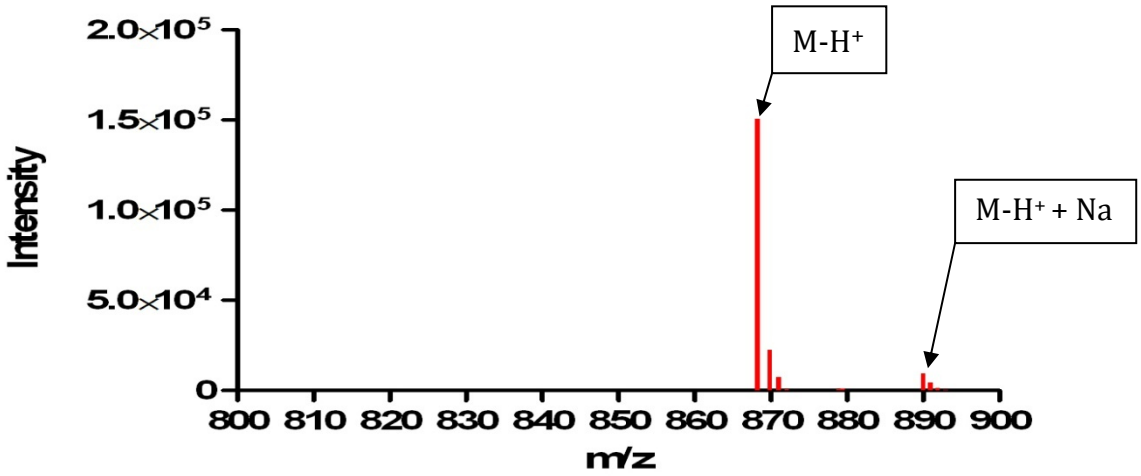


Figure 4: Mass spectrum of Folate PEG Biotin in Negative ion mode



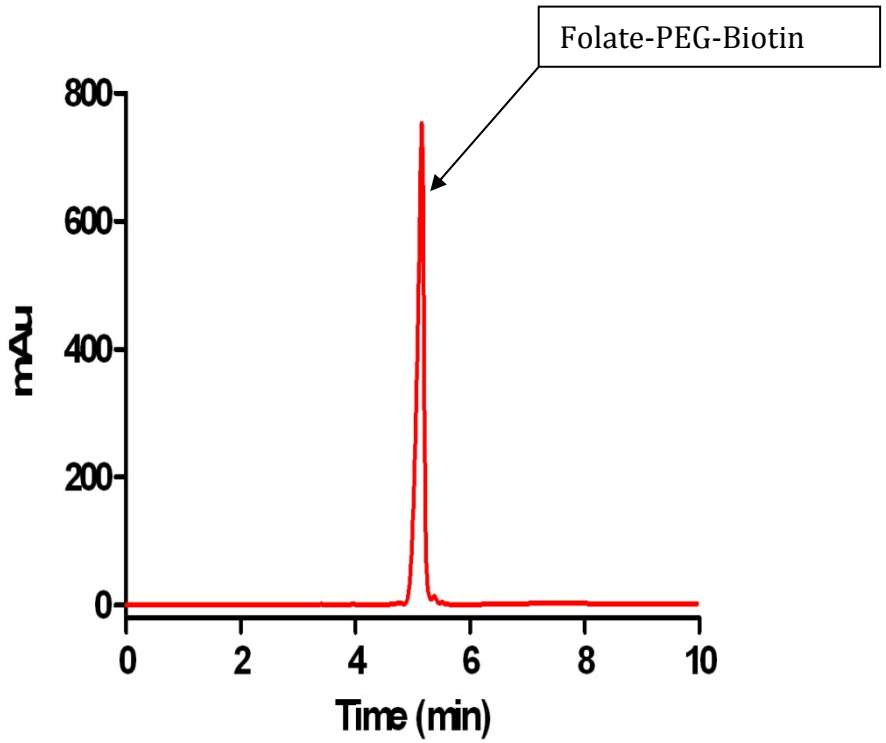


Figure 5: Cell Study

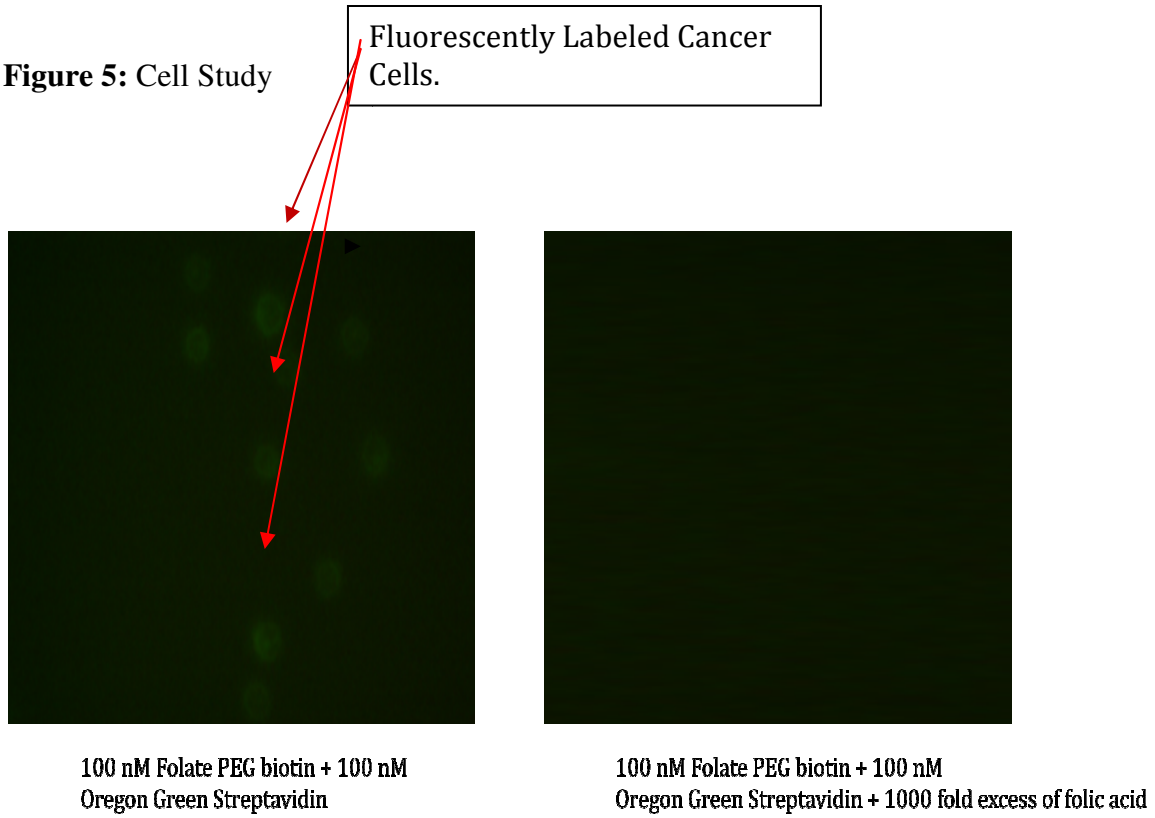
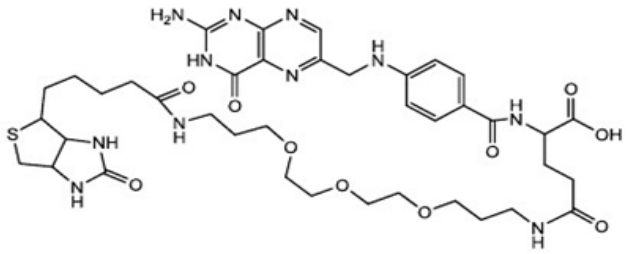


Figure 6:chemical structure of Folate-PEG-Biotin



Chemical Formula: $C_{39}H_{55}N_{11}O_{10}S$
Exact Mass: 869.39
Molecular Weight: 869.99
m/z: 869.39 (100.0%), 870.39 (43.2%), 871.39 (13.0%), 870.38 (4.9%), 871.38 (4.6%), 872.38 (2.1%), 872.39 (1.5%), 872.40 (1.3%)
Elemental Analysis: C, 53.84; H, 6.37; N, 17.71; O, 18.39; S, 3.69

Figure 7:HPLC (Hewlett Packard Series 1050) & Rigel HPLC C_{18} Column

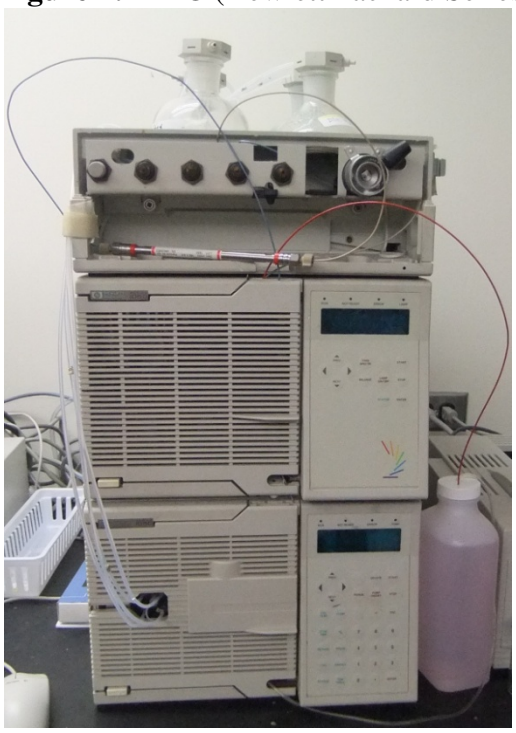


Figure 8: LC/MS & Agilent Eclipsed XDB C_{18} Column

Figure 9: Mini Centrifuge

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