Immunosome®-Biotinyl Cap (Non-PEGylated)

DESCRIPTION

Biotinylated liposomes can be conjugated noncovalently with (strept)avidin through either direct interaction with the protein/antibody conjugated to (strept)avidin or by coupling with other biotinylated proteins using (strept)avidin as a bridging molecule. Both avidin and streptavidin form strong noncovalent bond with biotin. The high resistance to breakdown makes them very useful in bioconjugate chemistry. However, streptavidin has replaced avidin in most bioconjugation applications due to its enhance properties. NeutrAvidin (ThermoFisher) is a modified avidin without negative properties. It performs much better than original avidin and sometimes streptavidin.

In order to exploit the high-affinity interaction of biotin with strept(avidin), a two-step "sandwich" protocol (Method A) has been developed for the preparation of targeted immunoliposomes. In this methodology, (strept)avidin is first attached to biotinylated liposomes, then a biotin-modified protein/antibody is introduced into the biotinylated strept(avidin)-labeled liposomes. This noncovalent approach is rapid, extremely versatile and applicable to numerous targeting ligands of interest with respect to *in vitro* and *in vivo* applications. Alternatively, instead of forming a strept(avidin) bridge, strept(avidin) molecule can also be covalently conjugated to antibody or ligand (Method B) and non-covalently bound to liposomes containing biotin on surface in order to form immunoliposomes.

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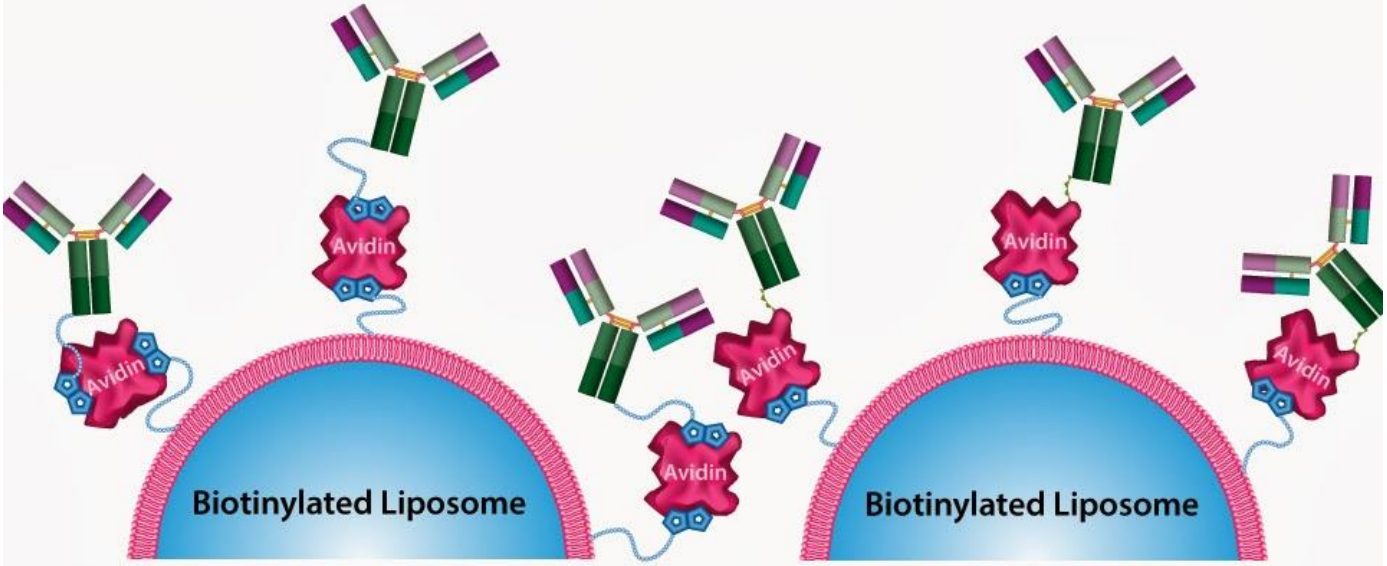
Antibody-Liposome Association Using Avidin-Biotin Binding



Both the liposome and the antibody may have conjugated biotin molecules. The quadrivalent avidin molecule "bridges" the two biotinylated components.

Alternatively, the antibody may be covalently bonded directly to the avidin which binds the biotin on the surface of the biotinylated liposomes.

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FORMULATION INFORMATION

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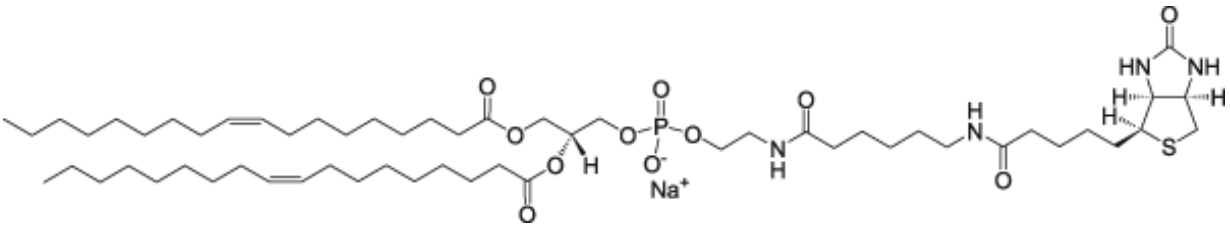
Lipid Composition	Concentration (mg/ml)	Concentration (mM)	Molar Ratio Percentage
L-α-Phosphatidylcholine	12	15.5	69
Cholesterol	2.6	6.73	30
1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine-N-(cap biotinyl)	0.25	0.22	1
			
Total	**14.85 mg/ml**	**22.45 mM**	**100**

Buffer and Liposome Size	Specification
Buffer	Phosphate Buffered Saline
pH	7.4
Liposome Size	100 nm

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CONJUGATION PROTOCOL

Materials and Equipment

In order to conjugate your antibody or protein tagged with biotin to Immunosome®-Biotinyl Cap liposomes you will need:

1. Laboratory magnetic stirrer is needed for dialysis.
2. Vortex laboratory mixer is recommended to have.
3. Float-A-Lyzer® with a proper MWCO that easily allows the cleanup of your liposome conjugated ligand from free and non-conjugated protein/peptide/ligand. You need to make sure that the MWCO is below 1,000,000 dalton. At 1,000,000 dalton, the pore size on the dialysis membrane gets close to 100 nm and therefore your liposomes can be dialyzed out. You cannot use dialysis cassettes blindly. Please understand the technique before using either spin column or dialysis cassette. If you do not use the correct MWCO you can lose your entire prep. For this protocol, we recommend MWCO of 300,000 dalton.

Preparation Method

Method A. Two-step "Sandwich" protocol; creating (strept)avidin bridge

1. The total lipid concentration in Immunosome®-Biotinyl Cap is 22.45 mM. 1% mol of the lipid in liposomes contains Biotinyl Cap group and only half of them are exposed to the outside of the liposomes, which is equal to 0.11 mM of reactive conjugable lipid. For 2 ml volume liposomes, this is equal to 2.20×10^{-7} mol, and for 5 ml volume liposomes, this is equal to 5.50×10^{-7} mol of Biotinyl Cap. Pour Immunosome®-Biotinyl Cap in a conical tube and vortex it gently with one hand. Use the other hand and slowly add the (strept)avidin solution until the two solutions are mixed. You need to use 10-fold molar excess of strept(avidin) to Biotinyl Cap lipid. Incubate the solution for 1 h at room temperature.

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2. Remove the unbound (strept)avidin from the prep by dialysis. We prefer dialysis to size exclusion columns. Dialysis is a much slower process but there will be minimum loss of Immunosome®-Biotinyl Cap after the prep is cleaned from unbound (strept)avidin. Spin columns are much faster; however, you can easily lose over 50% of the liposomes on the spin column. We recommend using Float-A-Lyzer® dialysis cassette with 300K MWCO from Spectrum Labs. Dialyze the Immunosome®-Biotinyl Cap/(strept)avidin solution in 1 liter of PBS at pH 7.4 for 8 hours. Change the dialysis buffer with a fresh 1 liter of PBS and let it dialyze for another 8 hours. After this step, Immunosome®-Biotinyl Cap/(strept)avidin is separated from unbound (strept)avidin.
3. Pour Immunosome®-Biotinyl Cap/(strept)avidin in a conical tube and vortex it gently with one hand. Use the other hand and slowly add the biotinylated antibody or biotinylated ligand solution until the two solutions are mixed. You need to use 2-fold molar excess of biotinylated antibody (ligand) to Biotinyl Cap lipid. Incubate the solution for 1 h at room temperature.
4. Remove the non-conjugated antibody or ligand from the prep by dialysis by using Float-A-Lyzer® dialysis cassette with 300K MWCO from Spectrum Labs. Dialyze the immunoliposome solution in 1 liter of PBS at pH 7.4 for 8 hours. Change the dialysis buffer with a fresh 1 liter of PBS and let it dialyze for another 8 hours. After this step, your cleaned up immunoliposome solution is ready to use.

Method B. Using an antibody/protein/ligand which is already covalently attached to (strept)avidin (less common method)

1. The total lipid concentration in Immunosome®-Biotinyl Cap is 22.45 mM. 1% mol of the lipid in liposomes contains Biotinyl Cap group and only half of them are exposed to the outside of the liposomes, which is equal to 0.11 mM of reactive conjugable lipid. For the 2 ml volume liposomes, this is equal to 2.20×10^{-7} mol, and for the 5 ml volume liposomes, this is equal of 5.50×10^{-7} mol of Biotinyl Cap. Pour Immunosome®-Biotinyl Cap in a conical tube and vortex it gently with one hand. Use the other hand and slowly add the antibody conjugated strept(avidin) until the two solutions are mixed. You need to

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use 2-fold molar excess of antibody conjugated strept(avidin). Incubate the solution for 1 h at room temperature.

2. Remove the non-conjugated antibody or ligand from the prep by dialysis by using Float-A-Lyzer® dialysis cassette with 300K MWCO from Spectrum Labs. Dialyze the immunoliposome solution in 1 liter of PBS at pH 7.4 for 8 hours. Change the dialysis buffer with a fresh 1 liter of PBS and let it dialyze for another 8 hours. After this step, your cleaned up immunoliposome solution is ready to use.

TECHNICAL NOTES

- To avoid precipitation of lipid in the noncovalent approach, care needs to be employed in maintaining a high ratio of strept(avidin) to Biotinyl Cap-liposomes. Otherwise, the coupling efficiencies would be relatively low.
- Alternatively, Sepharose® CL-4B size exclusion spin column can be used instead of Float-A-Lyzer®. However, keep in mind that a large amount of liposomes will be loss on the column during the process. Dialysis is a much slower process that size exclusion; however, there will be minimal loss of liposomes.
- If you decide to use a dialysis cassette, you will need to make sure that the MWCO is below 1,000,000 dalton. At 1,000,000 dalton, the pore size on the dialysis membrane gets close to 100 nm and therefore, your liposomes can be dialyzed out. You cannot use dialysis cassettes and spin columns blindly. They come in various sizes and you need to choose the correct size wisely.
- If you are using a ligand or peptide that is hydrophobic then it is recommended to solubilize it in DMSO or DMF and then add the buffer to it. It is recommended not to use more than 5% volume of DMSO or DMF in the solution. DMF and DMSO are both compatible with liposomes and they are also miscible in water. Other organic solvent such as ethanol and chloroform are not compatible with liposomes and will cause the liposomes to lyse. If you end up using DMSO or DMF then after the conjugation reaction is done, you need to remove DMSO and DMF from the liposomes. In order to do that you

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need to use a dialysis cassette that is made from REGENERATED CELLULOSE MEMBRANE. **NOTE**: Not all membranes are compatible with DMF and DMSO. We recommend using a Slide-A-Lyzer™ MINI Dialysis Device with MWCO of 2K made from regenerated cellulose membrane manufactured by ThermoFisher. After DMSO or DMF is removed, you can use Float-A-Lyzer® dialysis device for the final step of cleaning up the prep.

- Liposomes should be kept at 4 °C and **NEVER** be frozen.

APPEARANCE

Immunosome®-Biotinyl Cap is a white translucent liquid made of nano size unilamellar liposomes. Usually due to the small size of liposomes no settling will occur in the bottom of the vial. The liposomes are packaged in an amber vial.

STORAGE AND SHELF LIFE

Storage

Immunosome® products should always be stored at in the dark at 4 °C, except when brought to room temperature for brief periods prior to animal dosing. DO NOT FREEZE. If the suspension is frozen, the encapsulated drug can be released from the liposomes thus limiting its effectiveness. In addition, the size of the liposomes will also change upon freezing and thawing.

Shelf Life

Immunosome®-Biotinyl Cap is made on daily basis. The batch that is shipped is manufactured on the same day. It is advised to use the products within 4 months of the manufacturing date.

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REFERENCES AND BACKGROUND READING

1. *Hermanson GT. Bioconjugate techniques. Academic press; 2013 Jul 25*.

2. *Loughrey HC, Choi LS, Wong KF, Cullis PR, Bally MB. Preparation of streptavidin-liposomes for use in ligand-specific targeting applications. Liposome technology. 1993;3:163-78*.

3. *Haugland RP, Bhalgat MK. Preparation of avidin conjugates. Immunochemical Protocols. 1998:185-96*.

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