Immunoliposome preparation kit (-NHS, PEGylated)

DESCRIPTION

This product is used for the preparation of immunoliposome:

1, N-Hydroxysuccinimide (NHS) esters of DSPE-PEG-NHS react with the primary amine groups on the peptides, proteins, and antibodies.

2, The lipid conjugated ligand and non-reactive PEG lipids are mixed together to form micelles.

3, PEG micelles are post-inserted into the liposomes in order to form PEGylated ligand surface conjugated liposomes.

COMPONENTS

	Lipo-Im001RG-S, 2mL	Lipo-Im001RG-L, 5mL
Vial 1, Performed Liposomes(HSPC/CHOL, 60:40 molar ratio, 100nm)	Liquid, 1.6mL	Liquid, 4mL
Vial 2, DSPE-PEG(2000)-NHS lipid (Reactive lipid)	Powder, 3.34mg(0.55μmol)	Powder, 1.34mg(0.22μmol)
Vial 3, DSPE-PEG(2000) lipid (Non-reactive lipid)	Powder, 12.5mg	Powder, 5mg

PROTOCAL

Equipment & Reagent

1, Vortex mixer

2, Magnetic stirrer for dialysis

3, 10mL round bottom flasks

4, A rotary evaporator for making lipid film

(Alternatively, you can use a nitrogen tank connected to a thin hose for creating a stream of nitrogen flow to dry the lipid and make a thin film.)

5, Chloroform or methylene chloride

6, PBS Buffer

7, Bath sonicator or vortex

8, Dialysis membrane

Preparation Method

1, Dissolve the content of vial 3 in 100 μl (250μL)of chloroform or methylene chloride for 2mL-kit (5-ml kit size). Transfer the solution to a 10 ml round bottom flask. Dry the chloroform using a rotary evaporator or under a stream of nitrogen and make a dried lipid film.

2, Add 100 μl(250μL) of PBS buffer to the dried lipid film for 2mL-kit (5-ml kit size).

It is preferred to sonicate the hydrated lipid film using a bath sonicator and sonicate the micelle solution for 5 minutes. If you do not have a bath sonicator, then hydrate the dried lipid film with PBS for at least 1 hour and constantly rotate

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the solution in the round bottom flask using a rotavap (not connected to vacuum) or by hand to make sure that all the dried lipid on the wall of the round bottom flask will go to the solution and form micelles. Alternatively, you can use a vortex to agitate the solution. The goal is to have all the dried lipid on the wall of the round bottom glass to go the micelle solution. Cover the mouth of the round bottom flask with parafilm. Refrigerate the micelle solution of non-reactive PEG lipids until it is ready to be mixed with micelles formed in step 4.

3, For the 2-ml kit size, dissolve the content of vial 2 in 100 µl of chloroform or methylene chloride (250 µl for the 5-ml kit size). Transfer the solution to a 10 ml round bottom flask. Dry the chloroform using a rotary evaporator or under a stream of nitrogen.

4, Add the water-soluble protein, peptide or ligand at 1:2 molar ratio of ligand to dried film of DSPE-PEG-NHS. For the 2-ml kit add 300 µl of water-soluble solution of protein of ligand in PBS (pH 7.4) to the dried lipid film(750µl for 5-ml k). It is preferred to sonicate the hydrated lipid film using a bath sonicator and sonicate the micelle solution for 5 minutes. If you do not have a bath sonicator then hydrate the dried lipid film with PBS for at least 1 hour and constantly rotate the solution in the round bottom flask using a rotavap (not connected to vacuum) or by hand to make sure that all the dried lipid on the wall of the round bottom flask will go to the solution and form micelles. Alternatively, you can use a vortex to agitate the solution. The goal is to have all the dried lipid on the wall of the round bottom glass to go the micelle solution. The solution is incubated at room temperature for 6 hours and in refrigerator for 24 hours. The reaction is pH sensitive. Read the technical note below for more information.

5, Mix the micelles in step 2 to the micelles in step 4. The total volume of micelles for the 2-ml kit should be 400 µl and for the 5-ml kit should be 1000 µl.

6, To conduct post-insertion, the micellar dispersion is then co-incubated with preformed plain liposomes at 60 ℃ for 30 min.

7, Remove the non-conjugated protein, peptide or antibody from the immunoliposomes by dialysis. We prefer dialysis to size exclusion columns. Dialysis is a much slower process but there will be minimum loss of immunoliposomes after the prep is cleaned from non-conjugated protein/peptide/ligand. 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. You will need to choose a cassette with proper MWCO depending on the MW of your protein, peptide, antibody or antibody fragment. NOTE: 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. 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 is dialyze for another 8 hours. After this step, your cleaned up immunoliposome is ready to be used.

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- **NOTE**

1, Hydrolysis of DSPE-PEG-NHS in aqueous solutions competes with the primary amine reaction, resulting in the elimination of the NHS group, which can consequently decrease the coupling yield prior to the reaction with the protein/antibody. In order to minimize the impact of the hydrolysis of NHS ester, use a high concentration of protein/antibody to increase the efficiency of the crosslinking.

2, The reaction of NHS esters with amines is strongly pH-dependent: at low pH, the amino group is protonated, and no modification takes place. At higher-than-optimal pH, hydrolysis of NHS ester is quick, and modification yield diminishes. The half-life of NHS esters at pH 7 and 8 is 4-5 hours and 1 hour, respectively. Whilst NHS esters have a half-life of only 10 minutes at pH 8.6. Therefore, to avoid the hydrolysis of NHS ester, DSPE-PEG-NHS lipid should be used immediately for conjugation to antibodies, proteins or peptides containing free amines. NHS ester reactions are conducted in common buffers at pH 7-8.

3, Primary amine buffers such as Tris should NOT be used because they compete for reaction; however, in some procedures, it is useful to add Tris or glycine buffer at the end of a conjugation procedure to quench (stop) the reaction.

4, 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 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.

Creative Biostructure

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