

appropriate volume of 14.28 mM EDTA in PBS, pH 7.2 is added to 1 mg antibody to give an EDTA concentration of 1 mM.

**[0162]** 6 mg of 2MEA is dissolved in 100  $\mu$ l 1 mM EDTA in PBS, pH 7.2. 1  $\mu$ l of this 2MEA solution is added per 10  $\mu$ l of antibody solution. This solution is mixed and incubated in a waterbath at 37 deg for 90 min.

**[0163]** This solution is then passed through a PD10 column (pre-equilibrated with 1 mM EDTA in PBS, pH 7.2) and 500  $\mu$ l fractions collected. A sample from each fraction is taken and measured on UV spectrophotometer, with the absorbance at 280 nm used to quantify the protein found in each fraction. The fractions containing significant concentrations of protein are chosen and combined and remeasured on the UV spectrophotometer. This measurement is used to determine the antibody concentration using an extinction coefficient of the antibody of 1 mg/ml=1.4 absorbance units at 280 nm.

**[0164]** Binding of Maleimide-PEG2-Biotin to Antibody

**[0165]** Maleimide-PEG2-biotin is dissolved in 1 mM EDTA in PBS, pH 7.2 to give a 20 mM solution. An appropriate volume of this is added to the reduced antibody to give a 40 times molar excess of maleimide-PEG2-biotin over reduced antibody. This is then mixed and incubated for 3 hours at room temperature.

**[0166]** This is then passed through another PD10 column which has been pre-equilibrated with 1 mM EDTA in PBS, pH 7.2. 500  $\mu$ l fractions are collected and measured using the UV spectrophotometer at 280 nm. The fractions containing significant protein levels are chosen and combined. A sample of this solution is measured again at 280 nm by absorbance, and the concentration of antibody determined using the extinction coefficient of the antibody of 1 mg/ml=1.4 absorbance units at 280 nm. The number of biotins bound per antibody are then determined using the Pierce biotin quantification kit, according to the manufacturer's instructions.

#### Latex

**[0167]** Biotinylated Antibody 5A6 Binding to Latex

**[0168]** 1  $\mu$ m neutravidin coated latex is washed in 0.1% tween-20 in PBS, pH 7.2 (using centrifugation at 16100 $\times$ g for 3.5 min, 4 deg C.) and resuspended in the same at a concentration of 0.5% solids. Biotinylated antibody 5A6 is diluted to a concentration of 200  $\mu$ g/ml in 0.1% tween-20 in PBS, pH 7.2. An equal volume of 200  $\mu$ g/ml b5A6 is then added to 0.5% latex. This solution is mixed well and incubated for 2 hours at room temperature with shaking on a rotary mixer (30 rpm) in the dark.

**[0169]** The particles are then washed 4 times (using centrifugation at 16100 $\times$ g, 3.5 min at 4 deg C.) with an equal volume of PBS, pH 7.2 to remove any unbound biotinylated antibody and resuspended in PBS, pH 7.2 to give a latex concentration of 0.25% solids.

**[0170]** This will be referred to in the text as functionalized latex 1.

#### Paramagnetic Particles

**[0171]** Binding of Antibody to Particle

**[0172]** 200 nm streptavidin coated paramagnetic particles are washed (using a magnetic separator) in 0.1% tween in PBS, pH 7.2 and resuspended in the same to give a concentration of 0.5% solids. Biotinylated antibody 1H12 is diluted in 0.1% tween in PBS, pH 7.2 to give 50 $\mu$ g/ml. An equal volume of 0.5% paramagnetic particles and 50  $\mu$ g/ml bioti-

nylated antibody are combined, mixed and allowed to incubate for 70 min at room temperature, with shaking using a rotary shaker at 30 rpm.

**[0173]** The paramagnetic particles were then washed 4 times (using a magnetic separator) in an equal volume of 0.1% tween in PBS, pH 7.2 and resuspended in the same to give a concentration of paramagnetic particles of 0.5% solids. This will be referred to in the text as functionalized paramagnetic particles.

#### Preparation of Latex Particles Using Amine-SPDP Interactions

##### Antibody

**[0174]** Antibody Disulphide Bond Reduction for Binding to SPDP

**[0175]** Use undiluted antibody (5A6) stock at a concentration between 2 and 7 mg/ml. An appropriate volume of antibody stock is removed to give 100  $\mu$ g antibody. An appropriate volume of 14.28 mM EDTA in PBS, pH 7.2 is added to 100  $\mu$ g antibody to give an EDTA concentration of 1 mM.

**[0176]** 6 mg of 2MEA is dissolved in 625  $\mu$ l 1 mM EDTA in PBS, pH 7.2. 1  $\mu$ l of this 2MEA solution is added per 10  $\mu$ l of antibody solution. This solution is mixed and incubated in a waterbath at 37 deg for 90 min.

**[0177]** This solution is then passed through a Zeba spin desalting column (pre-equilibrated with 1 mM EDTA in PBS, pH 7.2) and the flow through collected. A sample of flow through is taken and measured on UV spectrophotometer, with the absorbance at 280 nm recorded. This measurement is used to determine the antibody concentration using an extinction coefficient of the antibody of 1 mg/ml=1.4 absorbance units at 280 nm.

##### Latex

**[0178]** Amine functionalized fluorescent latex is washed in 1 mM EDTA in PBS, pH 7.2 (using centrifugation at 16100 $\times$ g for 3.5 min, 4 deg C.) and resuspended in the same at a concentration of 0.5% solids.

**[0179]** SPDP is dissolved in an appropriate volume of DMSO to give 20 mM concentration SPDP. SPDP in DMSO is then added to the 0.5% latex to give 1 mM SPDP. This is mixed and incubated in the dark for 70 min with gentle shaking (30 rpm on rotary mixer). The latex is then washed 3 times with 2 $\times$  reaction volume of 1 mM EDTA in PBS, pH 7.2 (using centrifugation at 16100 $\times$ g for 3.5 min, 4 deg C.). The latex is then resuspended in the same in the appropriate volume to give a latex concentration of 0.5% solids. This gives latex that is bound to SPDP, ready for attachment of reduced antibody.

##### Binding of Reduced Antibody Latex with Bound SPDP

**[0180]** Reduced antibody 5A6 is diluted to 1 mg/ml in 1 mM EDTA in PBS, pH 7.2. 1 mg/ml antibody is then mixed with 0.5% latex bound to SPDP in a 1:1 ratio. This gives a binding mixture of 0.25% latex with 500  $\mu$ g/ml antibody in 1 mM EDTA in PBS, pH 7.2.

**[0181]** This binding reaction is incubated at room temperature in the dark for 19.5 hours and then washed 4 times with 1 $\times$  reaction volume of PBS, pH 7.2 (using centrifugation at 16100 $\times$ g for 3.5 min, 4 deg C.). The latex was then resuspended in PBS, pH 7.2 in the appropriate volume to give 0.25% solids.