Human ELISPOT Protocol
(short version; for details see mouse ELISPOT Assay Protocol)

1st/Coating: (Sterile) PBS + Ab 100µl/well, ON, 4°C, covered with plastic wrap
Wash: (Sterile) 3 x with 200µl/well PBS
Blocking: (Sterile) PBS + 1% BSA fraction V, minimum 1h at RT
Wash: (Sterile) 3 x with 200µl/well PBS
Cells: (Sterile) Isolate PBMC on ficoll gradients, wash 1x in autologous serum or PBS and 2x in media, count, adjust cell concentration to 3 x 10^6/ml (300,000/well). Plate 100µl/well. [24h pre-activated cells can be used at lower concentrations, e.g. 100,000/well]. Add 100µl/well additional media containing nothing/Ag/mitogen/drug etc. that you want to test. Agitate plate gently before placing in incubator to evenly distribute cells. Incubate 24h (IFN-γ, IL-2) or 48h (IL-4, IL-5) at 37°C in 7% CO₂/humidified incubator.

---------------------**(Non-sterile from now on)**------------------------
Wash: 3 x PBS and 3 x PBS-Tween (0.05%), 200/well
If cells are sticky, last wash can be dH₂O.

2nd/Detecting: Dilute Ab in PBS/Tween/BSA, 100µl/well ON at 4°C
Wash: 3 x PBS-Tween 0.05%, 200µl/well
Enzyme: Dilute streptavidin-HRP 1:2000 (or HRP-conjugated Ab) in PBS/Tween /BSA , add 100µl/well for 2h at RT.
Wash: 3 x 200µl/well PBS
Substrate: (Wear gloves) Dilute AEC stock 1:30 in 0.1M acetate buffer and filter through a 0.45µm filter to remove colored precipitates. Just before developing, add 30% H₂O₂ (1:2000) [protect H₂O₂ from light]. Add 200µl/well and observe for spot development maximum 1h at RT.
Wash: 3 x dH₂O, 200µl/well [can be done under running water] to stop reaction
Drying: Dry plates without lids in the UPRIGHT position ON at RT to prevent any remaining substrate caught beneath the well from soaking back through the membrane onto your spots.

Solutions Needed
AEC stock: 100mg in 10 ml DMF in glass container (dangerous! Use fume hood)
0.1M acetate buffer: 148 ml of 0.2M acetic acid [11.55ml glacial acetic acid/L of H₂O] in 352 ml 0.2M sodium acetate [27.2g/L H₂O] bring up to 1 L of H₂O. Adjust pH to 5.0. (sterile filter)
Complete medium: RPMI, pen/strep [100U/ml+100µg/ml], 2 mM L-glutamine, 10% NBS or FCS (Sterile filter)
PBS-Tween: 500µl Tween20 /L of PBS (0.05%)
PBS-Tween-BSA: 500µl Tween20 /L of PBS (0.05%) + 10mg BSA fraction V (1%) (sterile filter)
PBS-BSA: PBS + 1% BSA fraction V (sterile filter)