Exercise 1

Feb 28th 2025

Q1: Which one of the following options does **NOT** play a role in monoclonal antibody production by Hybridoma technology?

✔ A. Antigen-specific T cells

B. Antigen-specific B cells
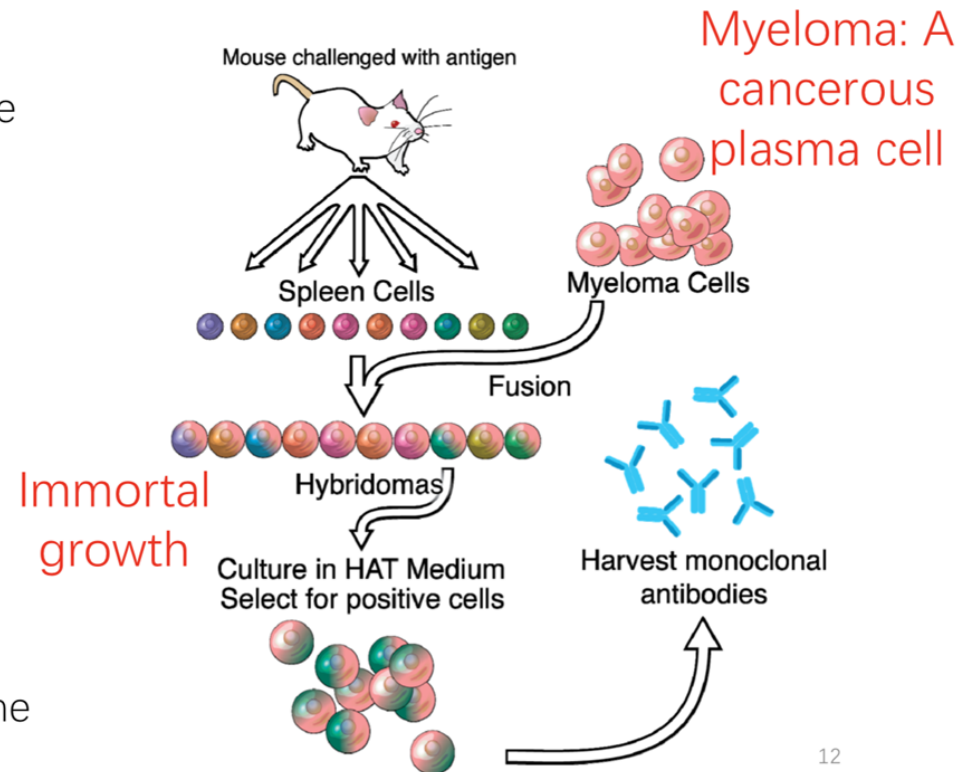
C. Myeloma cells

D. Culturing in HAT medium

A first transformative tool: Hybridoma technology – the generation of monoclonal antibodies

Recognition that the immune system of animal models could be used to generate **highly sensitive and specific reagents** for analyzing biological systems

Cesar Milstein and Georges Köhler at the time of their nobel prize award in 1984

Myeloma: A cancerous plasma cell

Mouse challenged with antigen

Spleen Cells

Myeloma Cells

Fusion

Immortal growth

Hybridomas

Culture in HAT Medium Select for positive cells
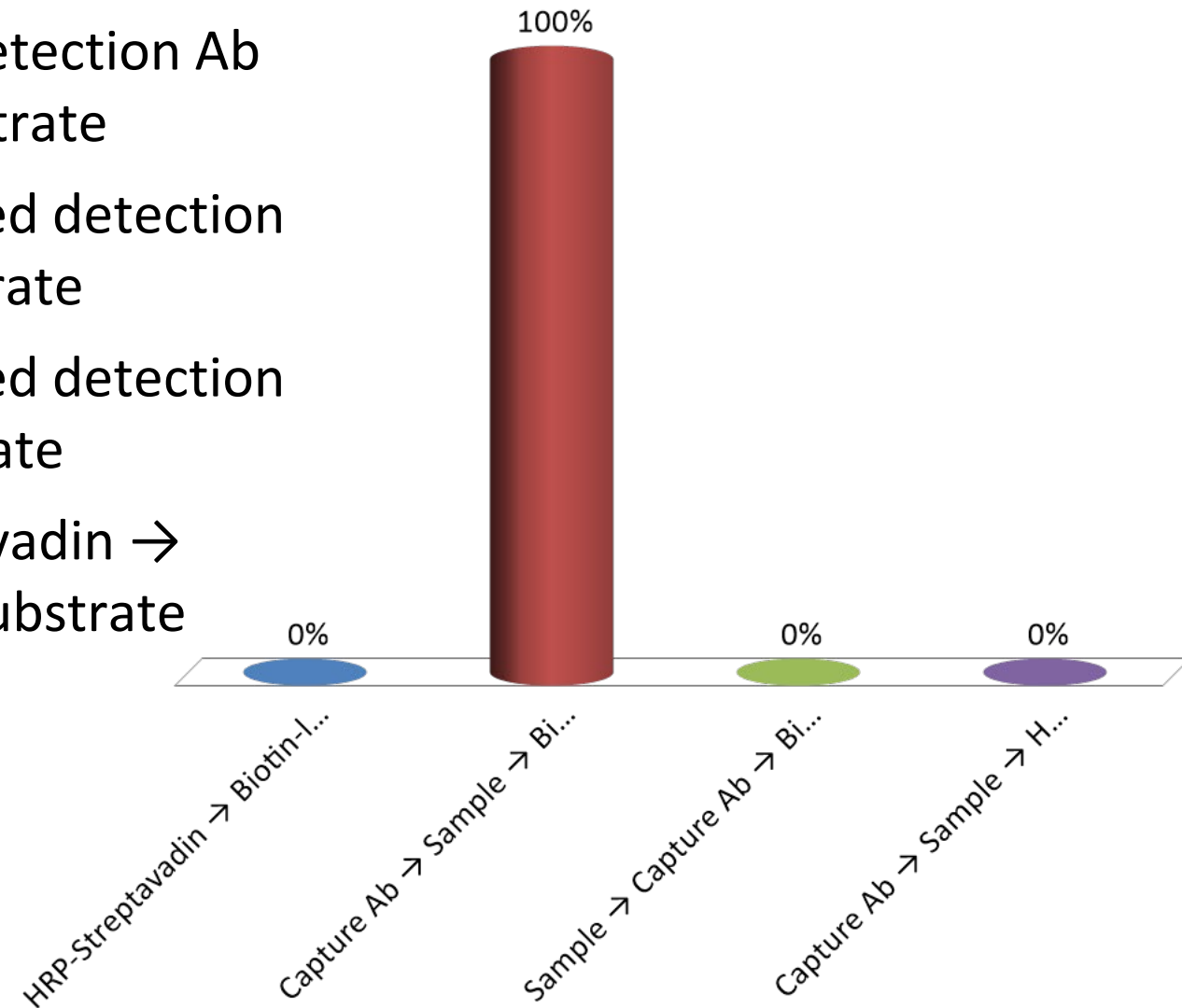
Harvest monoclonal antibodies

Q2: By using an ELISA kit for the detection of IFN-gamma among different treatments of T cells in one experiment, we will **NOT** be able to detect:...

A. The presence of IFN-y in different treatments

✔ B. The purity of IFN-y among other cytokines in different treatments

C. The quantity of IFN-y in different treatments
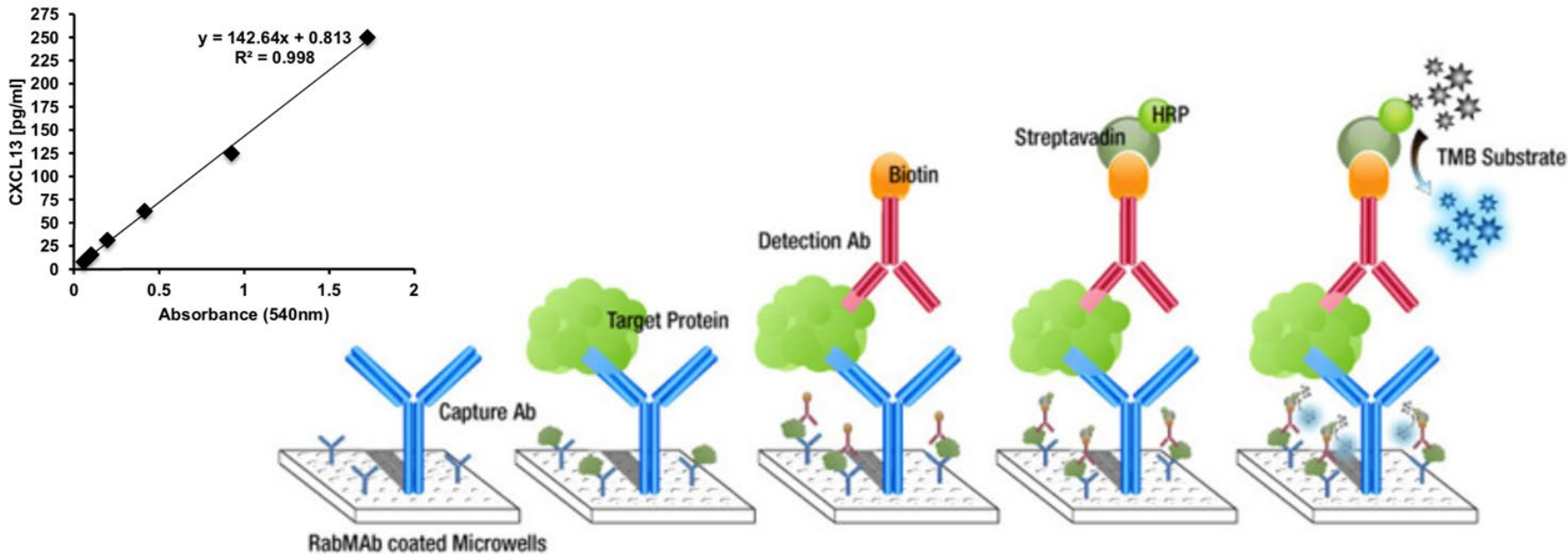
D. We can detect all above-mentioned options

Q3: To perform an ELISA, which options shows the correct sequence to add reagents to the immune-plate?

A. HRP-Streptavadin → Biotin-labelled detection Ab → Sample → Capture Ab → TMB substrate

✔ B. Capture Ab → Sample → Biotin-labelled detection Ab → HRP-Streptavadin → TMB substrate

C. Sample → Capture Ab → Biotin-labelled detection Ab-> HRP-Streptavadin → TMB substrate

D. Capture Ab → Sample → HRP-Streptavadin → Biotin-labelled detection Ab → TMB substrate

100%

0%   0%   0%

HRP-Streptavadin → Biotin-l...   Capture Ab → Sample → Bi...   Sample → Capture Ab → Bi...   Capture Ab → Sample → H...
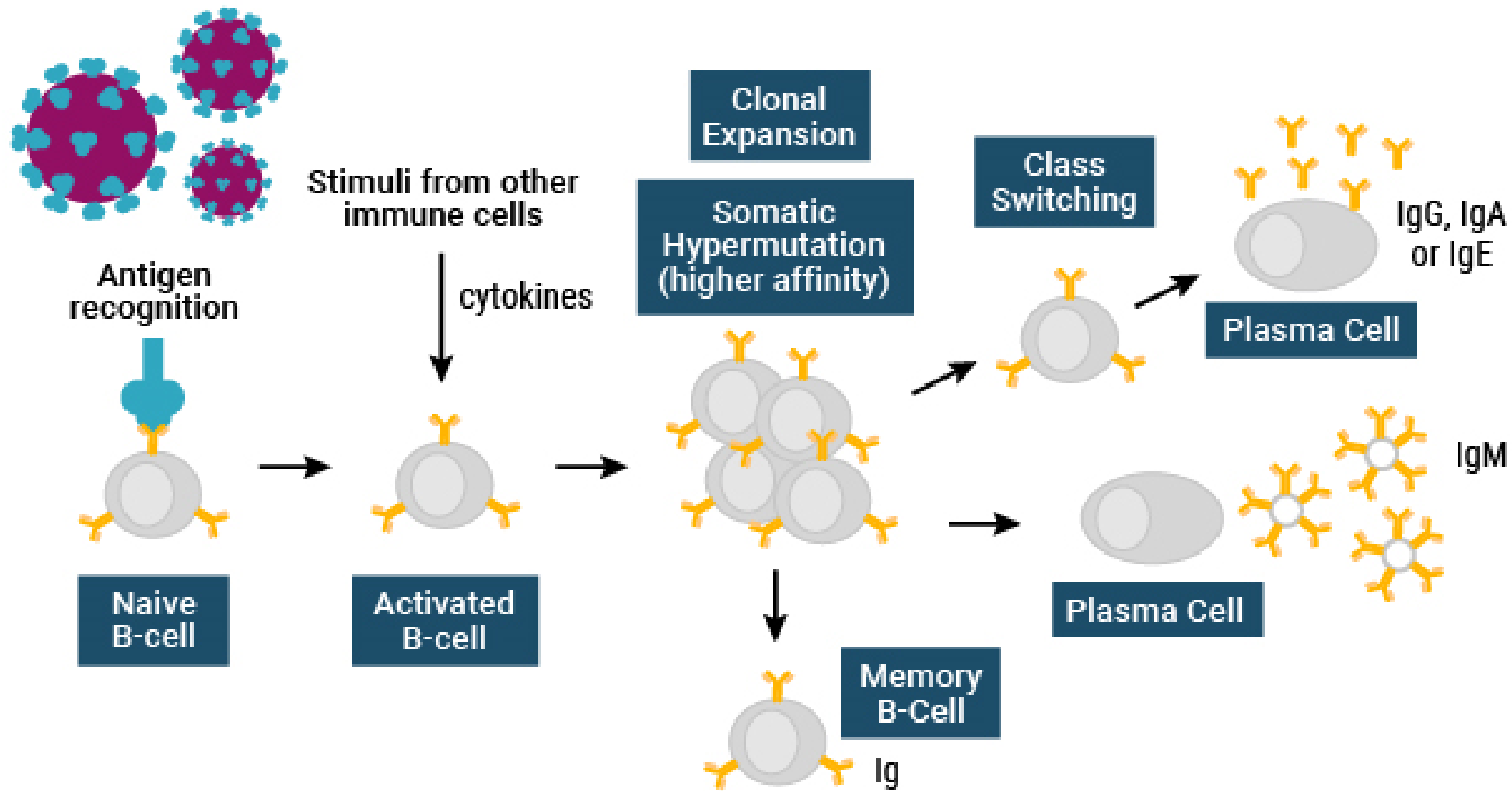
Putting monoclonal antibodies to work: Enzyme-linked immunoassay (ELISA)

Q4: Clonal expansion of OVA-specific B cells can be done by:

✓ A. Soluble ovalbumin

B. MHC I-bound ovalbumin peptides

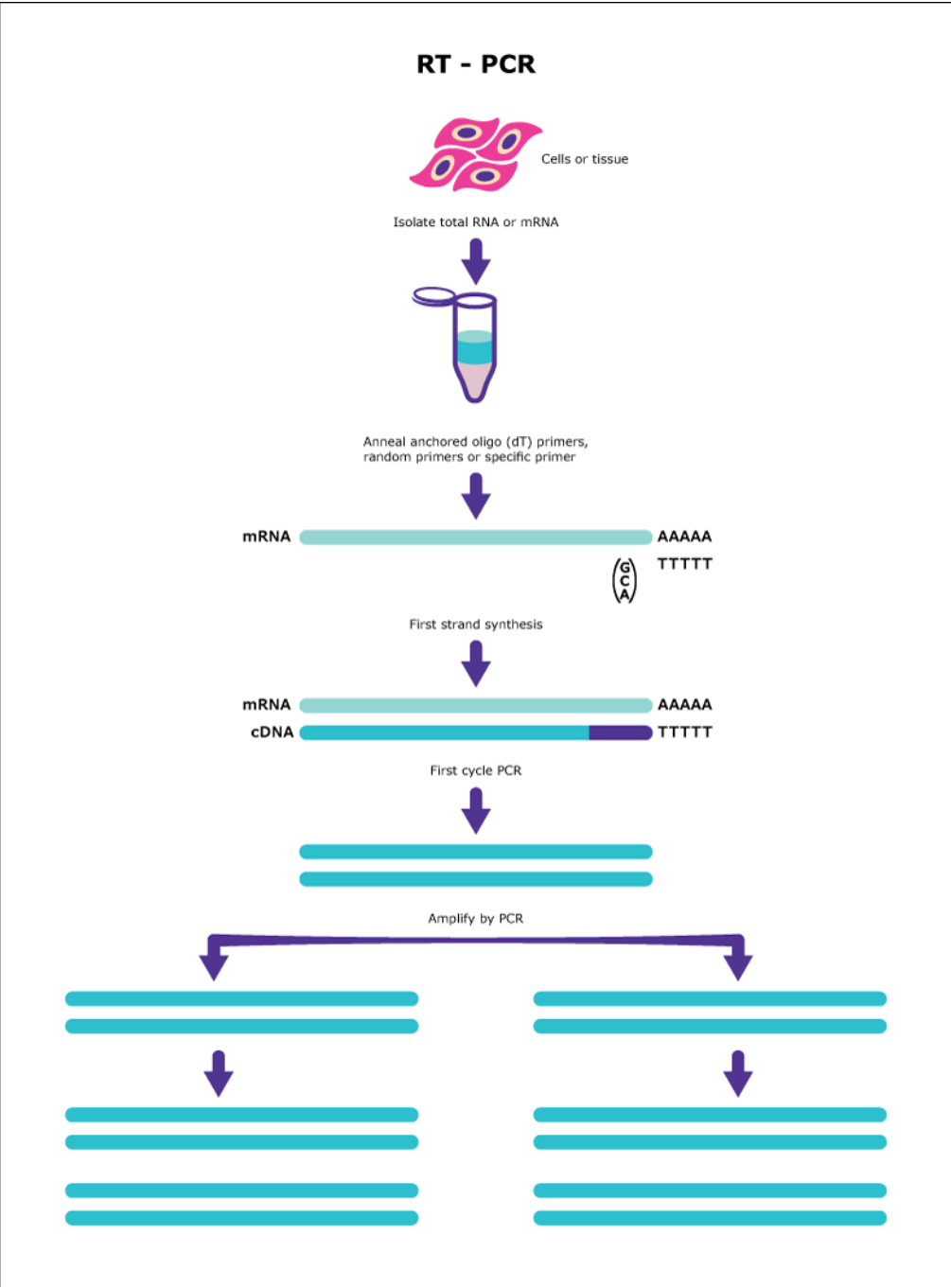C. MHC II-bound ovalbumin peptides

D. OVA-reactive T cells

Antigen recognition

Stimuli from other immune cells

cytokines

Clonal Expansion

Somatic Hypermutation (higher affinity)

Class Switching

IgG, IgA or IgE

Plasma Cell

Naive B-cell

Activated B-cell

Memory B-Cell

Ig

Plasma Cell

IgM

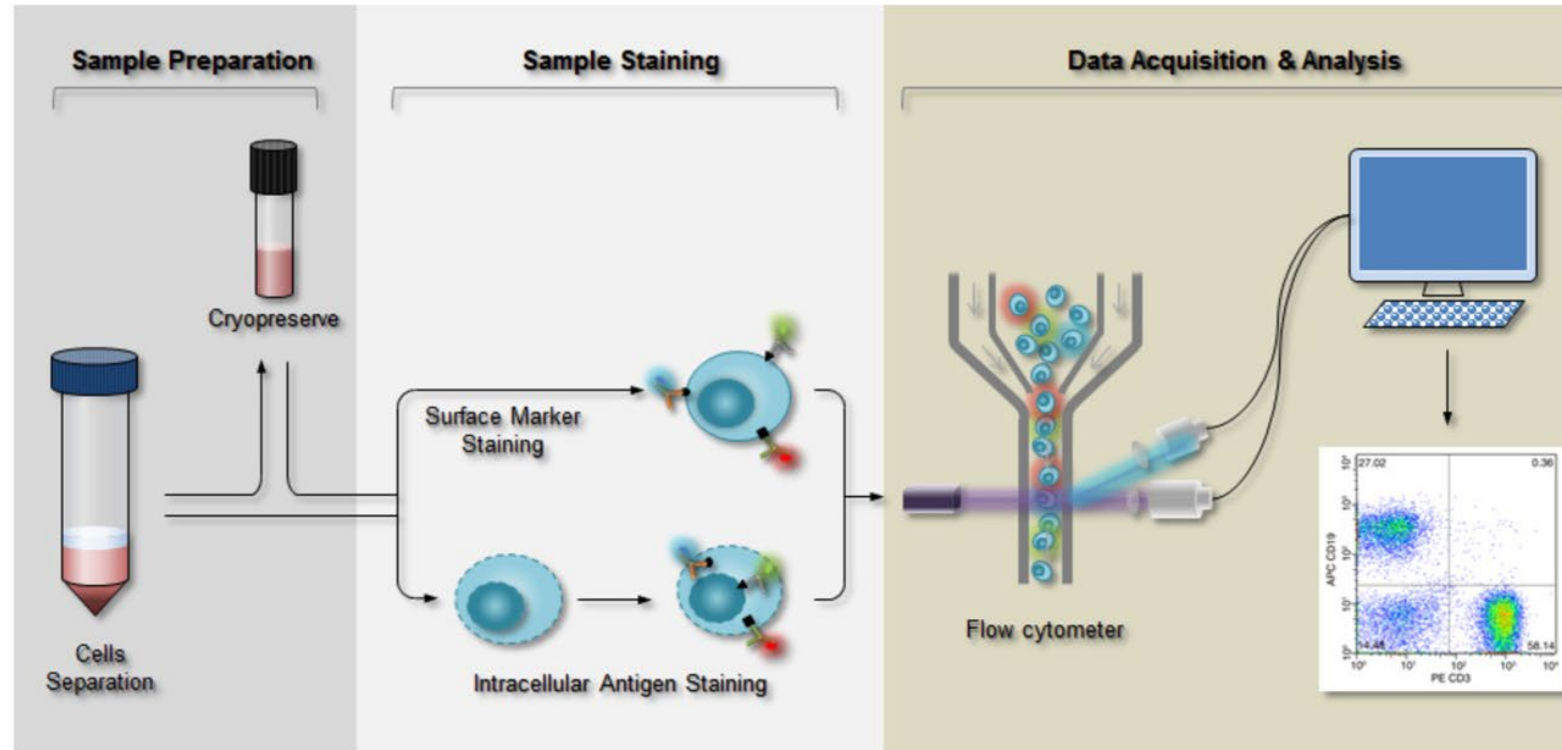# Q5: The transcript profile of T cells can be identified by:

✓ A. RT-qPCR

B. ELISPOT

C. Flow cytometry

D. Mass spectrometry

Reverse Transcription Quantitative
Polymerase Chain Reaction:

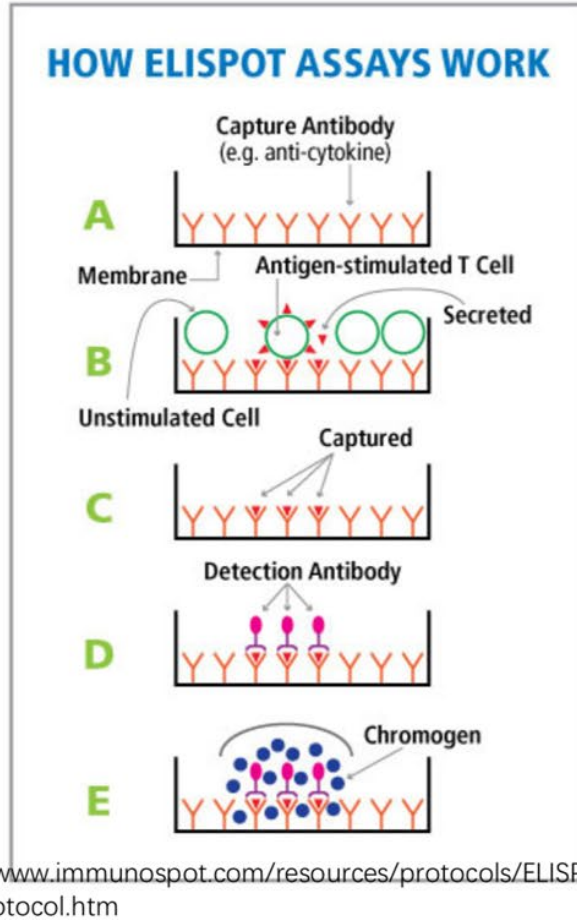**Quantify mRNA levels** in biological
samples

Sigma Aldrich

Flow cytometry
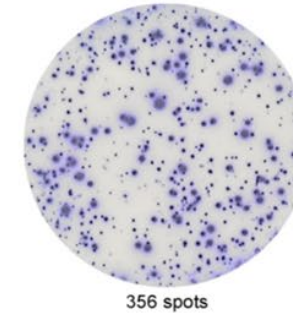
Identifying antigen-specific T-cells/B-cells

Ezyme-linked immunospot assay
(ELISPOT)

https://www.mabtech.com/knowledge-center/assay-principles/elispot-assay-principle/elispot-images

Limit of detection:
~0.0025% among assayed cells
(25 in 1,000,000)

http://www.immunospot.com/resources/protocols/ELISPOT-protocol.htm

18

Mass spectrometry

Q6: In a mixture of CD4+ T cells, CD8+ T cells and B cells, we can identify all T cells by the expression of:

✓ A. CD3

B. CD4

C. CD8

D. Tetramer