Protocol: Immuno-fluorescent Staining for Flow Cytometry

MATERIAL

- Round bottom test tubes (12x75mm)
- Purified Primary or Conjugated Primary antibodies
- If needed, conjugated secondary antibody
- Anti-CD16/CD32 for Fc-blocking for mouse tissues

BUFFERS

- Cell Staining Buffer: 1 X PBS, 5%FBS, 0.1% azide
- Sheath fluid for flow cytometer

INSTRUMENTS

- Pipettes and pipettors
- Ice bucket, refrigerator
- Centrifuge
- Flow cytometer

METHODS

I. Immunofluorescent staining of cell surface antigens for FACS Analysis

1. Harvest and prepare cells or tissues (spleen, lymph node, BM, thymus) as a single cell suspension*. Add cell staining buffer and centrifuge 4-5 min, 350 x g at 4°C and discard supernatant. If using splenocytes, perform an RBC lysis step and stop the reaction with cell staining buffer. (*Except for cultured cells, cells from tissues must be mechanically separated or by enzymatic digestion to produce single cell suspension.)
2. Wash cells with cell staining buffer. Count viable cells and resuspend at 1 x 10^6 cells/ml. Transfer 50µl to test tubes. Controls include: negative (no stain added), isotype control (with similarly labeled, non-specific primary antibody), and positive controls.
3. Blocking Fc Receptors is useful to eliminate non-specific immunofluorescent staining. For mouse cells, preincubate 0.5-1µg of anti-mouse CD16/CD32 per one million cells for 5-10 min on ice prior to staining blocks non-specific staining.
4. Add the optimally diluted conjugated, purified, or biotinylated primary antibody. Incubate on ice for 15 to 30 min in the dark.
5. Wash 3X with 2ml cell staining buffer, spin for 5 min (300-400 x g) at 4°C.
6. Resuspend cells in 500µl cell staining buffer and analyze cells.
7. If using purified or biotinylated antibodies, add the appropriate second step reagent in 100µl of staining buffer. Incubate at 4°C in the dark for 15-30 min. Repeat steps 5 and 6.

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8. Add viability dye to identify dead cells. Cells must be > 90% viable.

II. Intracellular staining for FACS analysis

1. Prepare cells to be analyzed. Stain cell surface antigens if required. Proceed with cell surface antigen staining protocol as listed above.
2. Add 100µl of fixative (1X PBS, 4% paraformaldehyde), incubate at 4°C for 15 to 30 min. Spin and decant supernatant carefully.
3. Add 100µl of permeabilizing buffer (1X PBS with 0.5% saponin) buffer and add the flurochrome-conjugated primary antibody. Incubate at 4°C for 30 min in the dark. Treat permeabilized cells gently, spin and decant.
4. Wash 2X with cell staining buffer. Spin cells and decant supernatant each time.
5. Resuspend cells in 500µl cell staining buffer and analyze on flow cytometer.

Note: The fixation and permeabilization treatment will significantly change the FSC/SSC distribution compared to live cells.

ABOUT

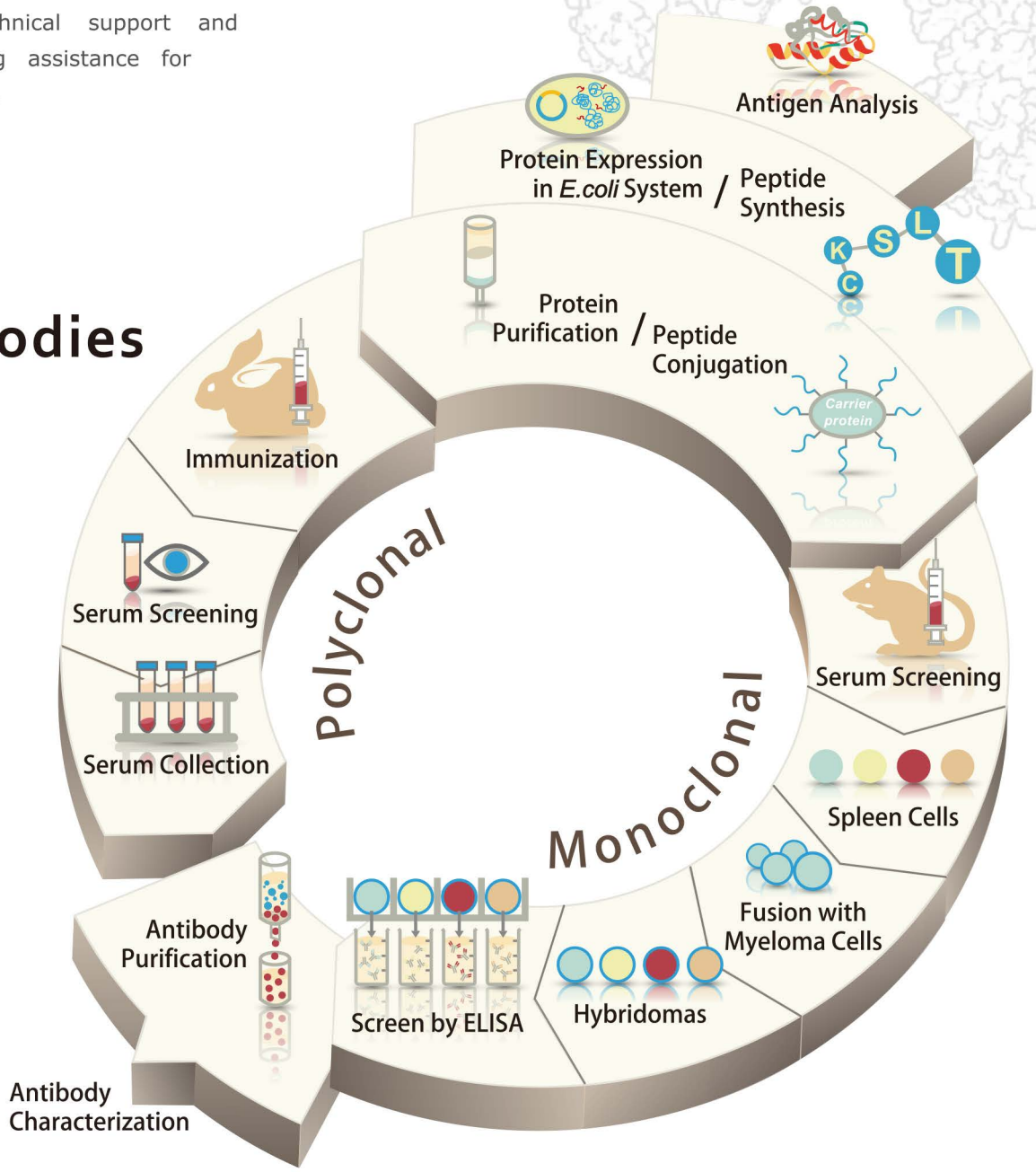
GeneTex

Starting as a small scientist-founded company in San Antonio, Texas, GeneTex has grown to become one of the top antibody manufacturers and has solidified its position in the life sciences industry.

Our corporate mission, to provide our customers with quality reagents and to accelerate life sciences research, reflects the philosophy and approach we employ when we manufacture our products. Through extensive research, development, and quality-assurance testing, we have produced and validated a comprehensive collection of antibodies and research reagents.

Due to GeneTex's experience in antibody production, we are uniquely capable of providing technical support and troubleshooting assistance for our customers.

Our Antibodies



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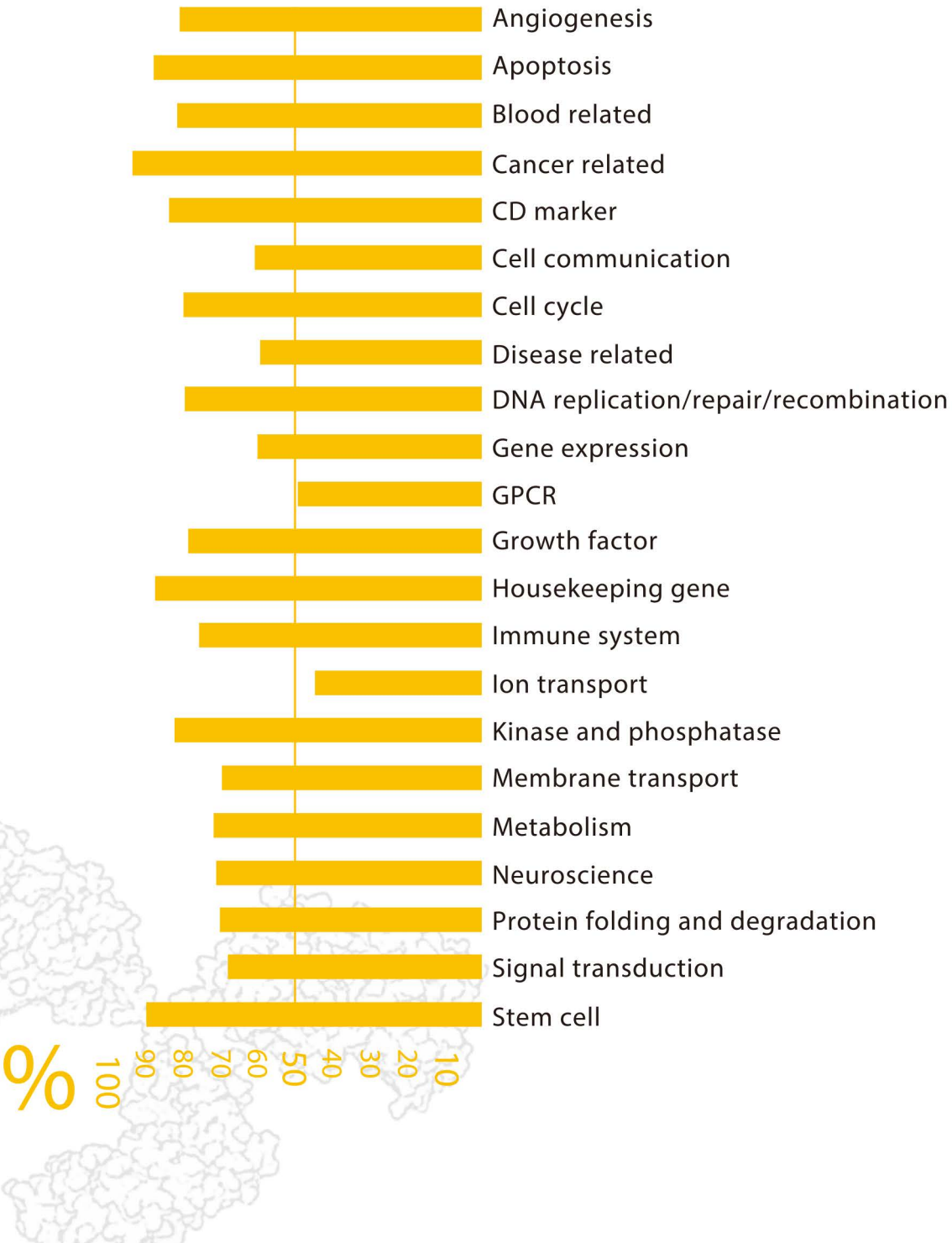
Selection you need

Comprehensive Coverage

GeneTex currently offers more than 55,000 products including over 45,000 primary antibodies that cover eleven different research areas. We continue to build upon our product portfolio to ensure that novel targets are available to researchers and that GeneTex keeps pace with new scientific discovery.

In addition to our comprehensive catalog of primary antibodies, GeneTex also offers a wide variety of quality reagents, kits and services.



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