Protocol: Immunocytochemistry (ICC)

MATERIAL

- Coverslip
- 24-well plate
- Primary antibody
- Conjugated secondary antibody
- ABC reagents
- Poly-L-Lysine (optional)
- DAPI
- Hematoxylin.

BUFFERS

- PBS
- Blocking buffer: 10% serum-PBS

METHOD

I. Pre-treatment of Coverslips

1. Dip the new bought cover slips into 0.1N HCl solution, and keep at RT overnight.
2. Wash with three changes of water to eliminate remaining HCl.
3. Dip the coverslips into 95% ethanol, and keep at RT overnight.
4. Drain all of the solution and air dry the coverslips.
5. Autoclave or fire burn (immediately before using) the coverslips.

II. Pre-coat of Coverslips (optional)

1. Dissolve 5mg poly-L-Lysine (PLL, Sigma P1274) with 50ml tissue culture grade water.
2. Cover each of coverslips with ~30µl PLL solution and stand for 5 min.
3. Remove the solution from coverslips and air dry at least 2 hrs before introducing cells and medium.

III. Cell seeding, Fixation and Permeability

1. Place the coverslip on to a 24-well plate. Seed about 25,000 cells into the well to reach approximately ~60-70% confluent next day.
2. Remove the medium and rinse cells twice with PBS.

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3. Add 1.0ml of -20˚C pre-cold methanol into the well and store the plate at -20˚C for 5-10 min.
4. Remove the methanol and add 1.0 ml PBS carefully into the well and gently shake for 10 min
5. Add 0.5ml of 0.05% Saponin for 15 min at RT.
6. Wash cells with PBS three times, each for 3 min.

IV. Primary / Secondary Antibody Incubation

1. Block the cells with blocking buffer for 30 – 60 min (serum from the same species as the secondary antibody is preferred. For example, if your secondary antibody is goat anti-rabbit antibody, you may want to use goat serum for blocking).
2. Dilute the primary antibody to desired dilution in blocking buffer (1 µl antibody to 200 µl serum-PBS if 1:200 is the case).
3. Add 200µl of primary antibody solution into each of the wells and incubate in RT for 1 hr or 4˚C overnight (recommended).
4. Wash cells with PBS three times, each for 3 min.
5. Add 200µl of secondary antibody (diluted in PBS) and incubate in RT for 30 -60 min.
6. Wash cells with PBS three times, each for 3 min.
7. Note: Keep the whole plate in the dark during step 5 and 6 if you are doing immunofluorescence.
8. If you are doing immunocytochemical staining, present the target protein by staining with appropriate kits, such as an ABC kit.

V. Nuclear staining and Mounting

1. If you are doing immunofluorescence, counter-stain the nuclear with 1: 500,000 ~ 1: 1,000,000 DAPI for 5 min or a shorter period of time. Rinse the cells with PBS twice and then mount the coverslip onto the slide with anti-fade mounting solution.
2. If you are doing immunocytochemistry, Counter-stain the nuclear in hematoxylin. Rinse the cells with PBS twice and then mount the coverslip onto the slide.

ABOUT

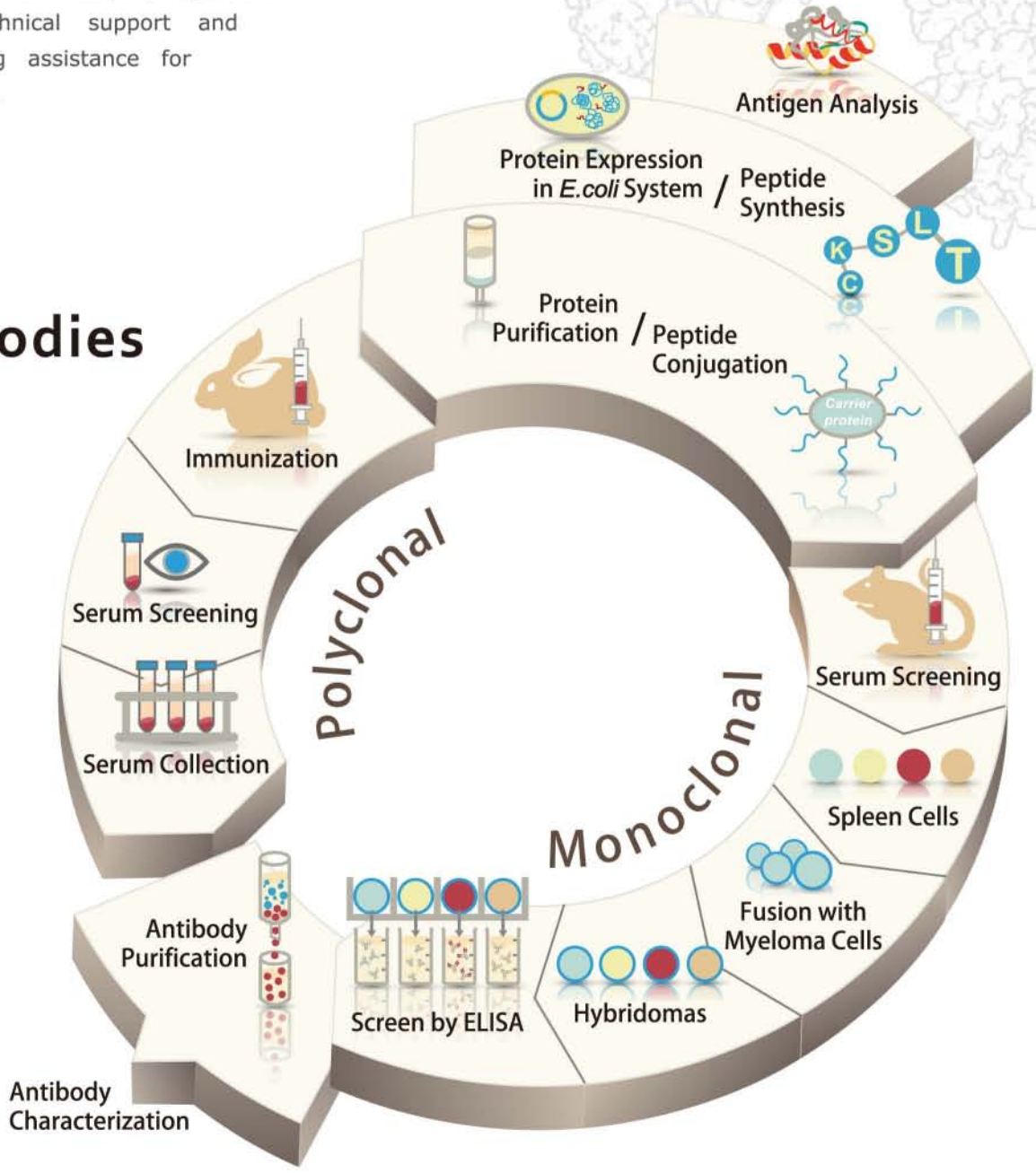
GeneTex

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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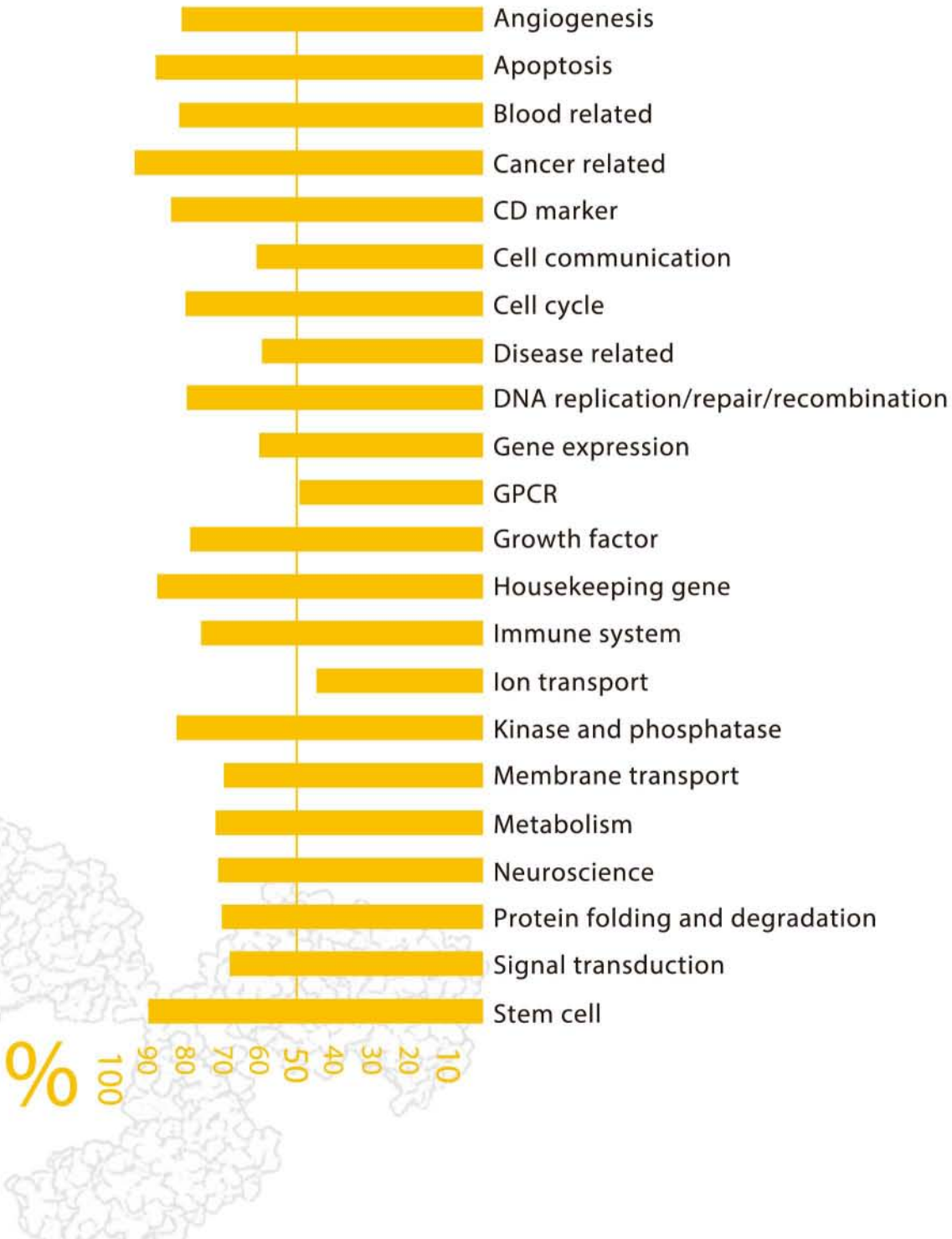
Selection you need

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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