Protocol: Immunocytochemistry (ICC)

MATERIALS/ REAGENTS/ BUFFERS

For tissue preparation and cryosectioning

- 0.1N HCl
- Ethanol
- ddH_2O
- Coverslips
- 24-well plate
- Methanol
- 4% Paraformaldehyde (PFA)
- Primary antibodies
- Conjugated secondary antibodies
- Coating solutions: 0.01% Poly-L-Lysine, or 0.1% gelatin
- DAPI (GTX16206) or Fluoroshield™ with DAPI (GTX30920)
- PBS
- Blocking buffer: 5% serum or 3%BSA in PBS

Protocol

I. Coverslips preparation

1 Immerse the newly bought coverslips into 0.1N HCl solution, and keep at room temperature overnight.

2 Wash with ddH_20 three times.

3 Immerse the coverslips into 95% ethanol, and keep at room temperature overnight.

4 Drain off excess ethanol, and air-dry the coverslips.

5 Autoclave for later use, or flame the coverslips for immediate use.

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II. Coverslips coating (optional)

For poly-L-Lysine coating:

1 Coat coverslips with 0.01% poly-L-lysine for 1 hour at room temperature.

2 Remove coating solution and air dry the coverslips for at least 2 hours before seeding cells.

For gelatin coating:

1 Coat coverslips with 0.1% gelatin for 20 minutes at room temperature.

2 Remove gelatin and rinse with PBS.

III. Cell seeding, fixation, and permeabilization

1 Place the coverslip in wells of a 24-well plate. Seed cells into wells and culture until cells reach desired confluency.

2 Remove culture medium and rinse cells twice with PBS.

3 Fix cells with methannol [3-1] or 4% paraformaldehyde/PBS (4% PFA) [3-2].

- 3-1 Fix cells with 1ml/well of -20°C pre-chilled methanol at -20°C for 5-10 minutes. Remove methanol and wash cells once with PBS. (Proceed to step 5.)
- 3-2 Fix cells with 0.5ml/well of 4% PFA at room temperature for 10 minutes. Remove PFA and wash cells once with PBS.

4 Permeabilize cells with 0.1 - 0.25% Triton X-100/PBS (or 0.5% saponin/PBS) at room temperature for 15 minutes.

Note: Skip this step if fix cells with methanol.

5 Wash cells with PBS three times, each for 3 minutes.

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IV. Blocking and immunostaining

1 Incubate the cells with blocking buffer (5% serum or 3%BSA in PBS) at room temperature for 1 hour.

Note: We recommend using serum from the species the secondary antibody was raised in.

2 Dilute the primary antibody in the blocking buffer according to its datasheet.

3 Incubate the cells with primary antibody solution at room temperature for 1 hour, or at 4°C overnight (recommended).

4 Wash cells with PBS three times, each for 3 minutes.

5 Incubate cells with secondary antibody diluted in the blocking buffer at room temperature for 1 hour.

Note: Keep cells in dark from this step if using fluorophore-conjugated secondary antibodies.

6 Wash cells with PBS three times, each for 3 minutes.

V. Counterstaining and mounting

1 Mount the coverslips onto slides with Fluoroshield™ with DAPI anti-fade mounting medium (GTX30920) to stain DNA.

2-1 Alternatively, incubate cells in 0.1-1 µg/mL Hoechst or DAPI (GTX16206) for 5 minutes.

2-2 Rinse cells with PBS twice before mounting.

2-3 Mount the coverslips onto slides with mounting medium (GTX28214).

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ABOUT

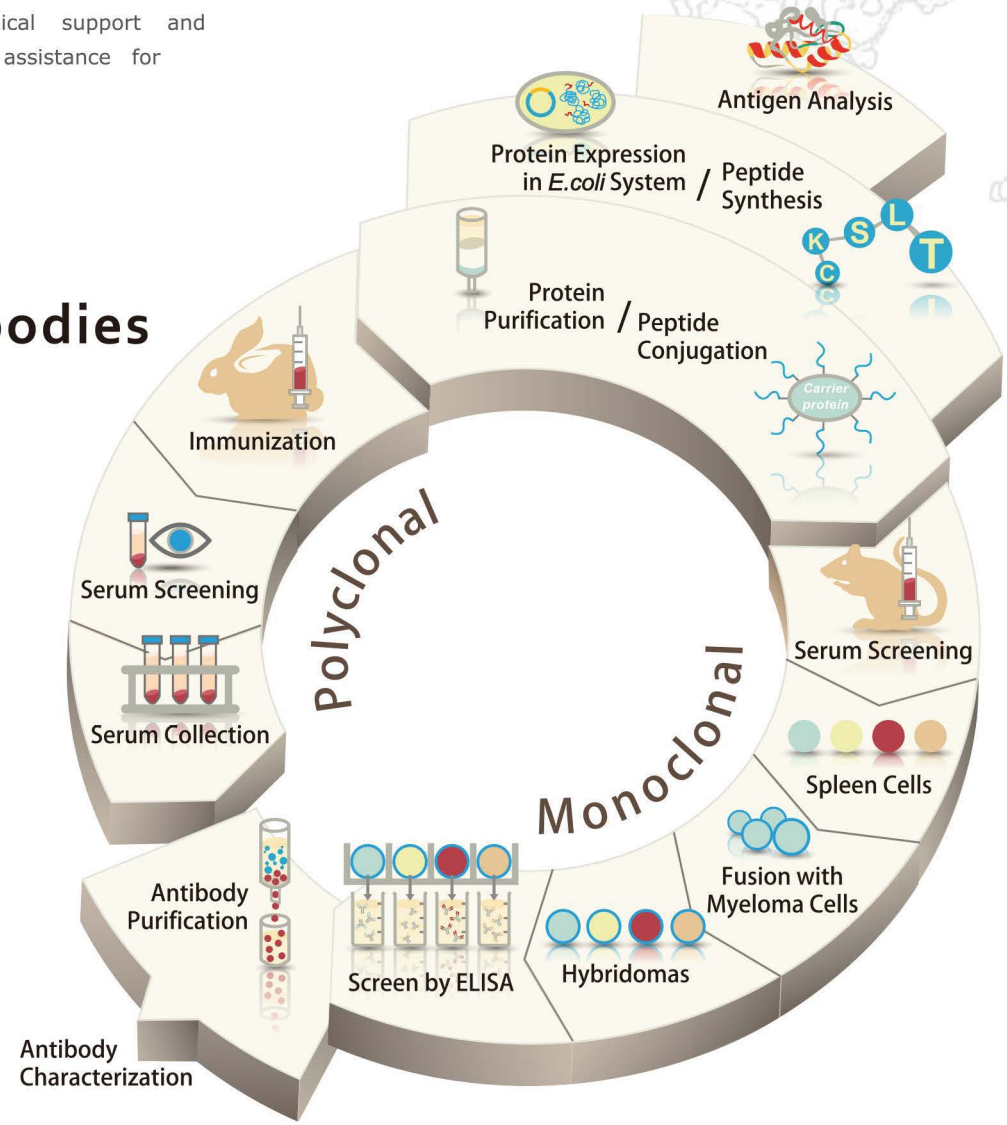
GeneTex

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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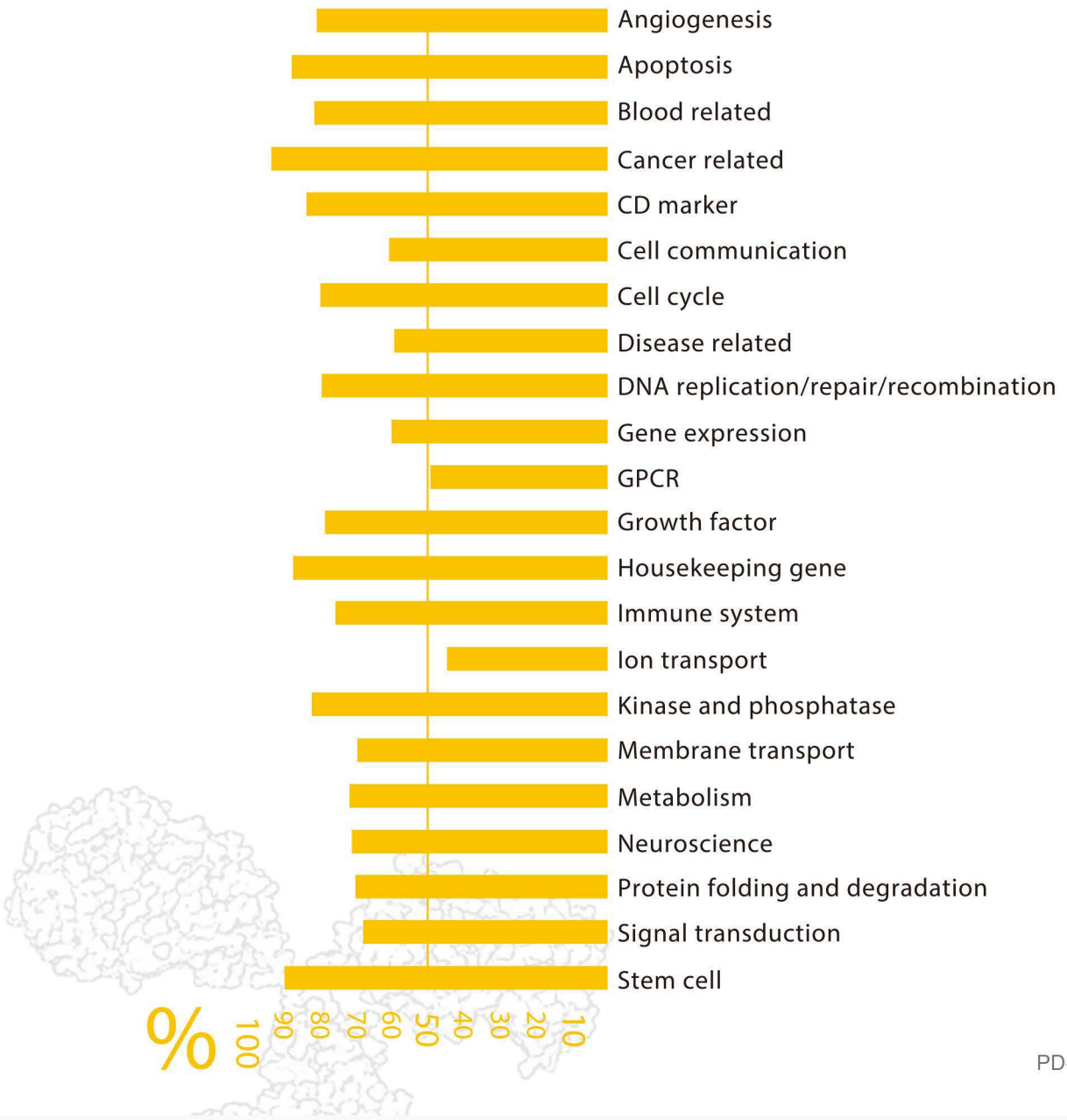
Selection you need

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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