Protocol: Immunoprecipitation

MATERIAL

- Cell lysate (refer to “Protocol: Cell Lysate Preparation”)
- Protein A or Protein G slurry
- Primary antibody

BUFFERS

- Lysis buffer
- PBS
- SDS-PAGE sample buffer

INSTRUMENTS

- Centrifuge
- Rocking platform or rotator

METHOD

I. Cell Lysate Preparation

Refer to the protocol for Cell Lysate Preparation.

II. Cell Lysate Preclearing

1. Transfer 50µl of the Protein G beads slurry (commercially available from several vendors) to a microcentrifuge tube and add 450µl cold Lysis Buffer. Spin at 10,000 x g for 30 seconds and remove the Lysis Buffer. Wash one more time with 500µl of cold Lysis Buffer. Resuspend the beads in 50µl of cold Lysis Buffer.
2. Add this 50µl of prepared Protein G slurry and 500µl of Cell Lysate to a microcentrifuge tube and incubate on ice for 30-60 min.
3. Spin at 10,000 x g for 10 min at 4˚C and transfer the supernatant to a new microcentrifuge tube. If any beads are transferred, spin again and carefully retransfer the supernatant to a new microcentrifuge tube.

III. Immunoprecipitation

1. Add 5-10µg of antibody to the microcentrifuge tube containing the cold precleared lysate. Note: This concentration of antibody is suggested as a starting point. Each investigator may desire to titrate the concentration of antibody and volume of cell lysate in preliminary experiments to determine the optimal conditions.
2. Incubate at 4˚C for 1 hr.
3. Add 50µl of washed Protein G slurry in prechilled Lysis Buffer (prepared as instructed in Preclearing Step 1 above).

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4. Incubate for 1 hr at 4˚C on a rocking platform or a rotator.
5. Spin the microcentrifuge tube at 10,000 x g for 30 seconds at 4˚C.
6. Carefully remove supernatant completely and wash the beads 3-5 times with 500µl of Lysis Buffer. To minimize background, care should be taken so that the supernatant is completely removed in these washes.
7. After the last wash, aspirate the supernatant and add 50µl of 1X SDS-PAGE sample buffer to bead pellet. Vortex and heat to 90-100˚C for 10 min.
8. Spin at 10,000 x g for 5 min, collect supernatant and load onto the gel. Supernatant samples can be collected and kept frozen at this point if the gel is to be run later.
9. Follow manufacturer's instructions for SDS-PAGE. Stain the gel for visual analysis of the immunoprecipitated protein. If using Western blot after this step, follow the accompanying Immunoblotting (WB) Protocol. Preferably, the antibody used for the immunoprecipitation portion is not the same antibody used for the western blot portion. A different clone with the same specificity is recommended.

IV. Binding Characteristics of Some Immunoglobulins

Immunoglobulin	Protein A	Protein G
Mouse IgG1	+	++
Mouse IgG2a	+++	+++
Mouse IgG2b	++	++
Mouse IgG3	+	+++
Mouse IgM	-	-
Mouse IgA	-	-
Mouse IgE	-	-
Rat IgG1	+	+
Rat IgG2a	-	+++
Rat IgG2b	-	++
Rat IgG2c	+	++
Human IgG1	+++	+++
Human IgG2	+++	+++
Human IgG3	-	+++
Human IgG4	+++	+++

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ABOUT

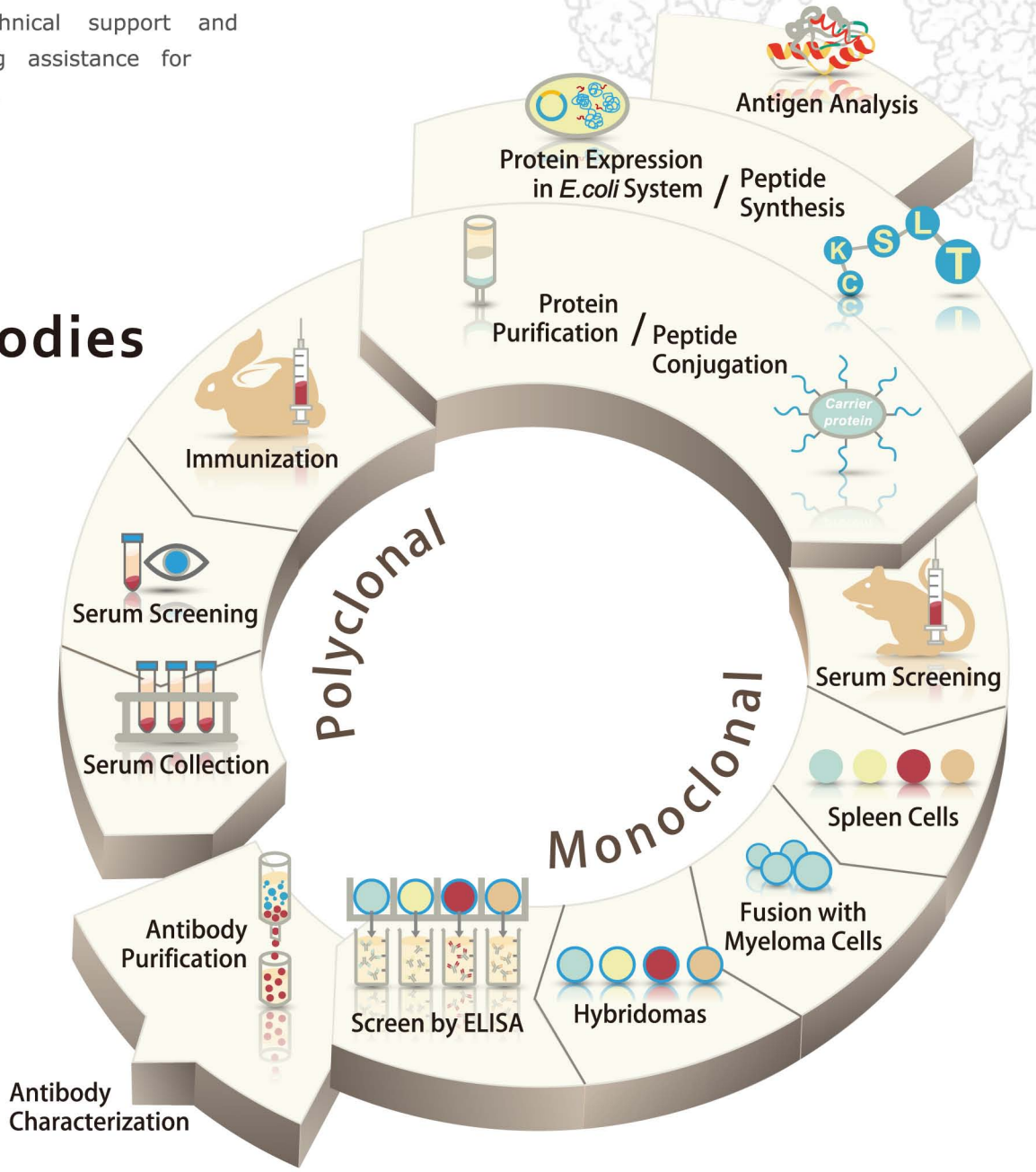
GeneTex

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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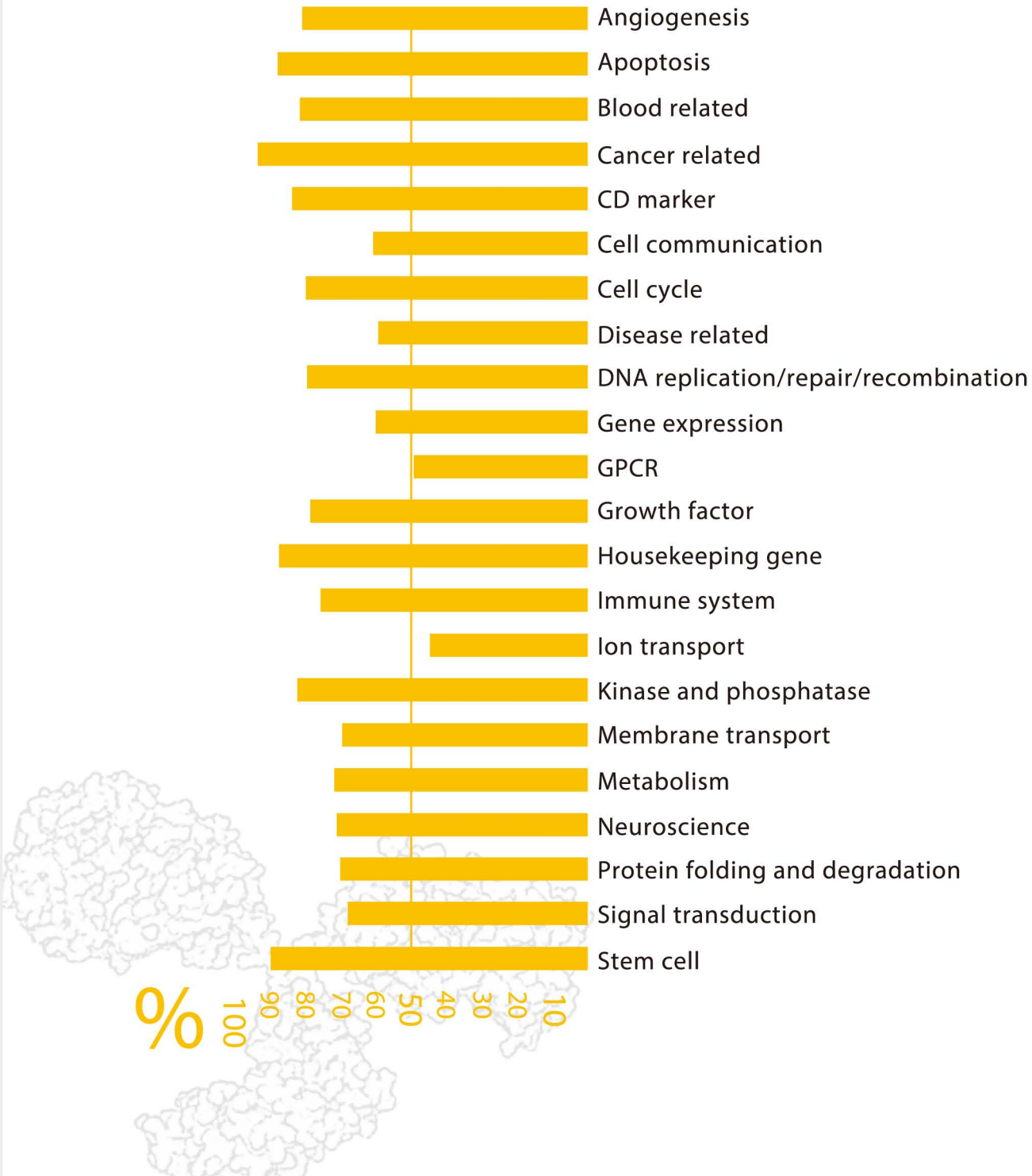
Selection you need

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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