Protocol: Cell Lysate Preparation

REAGENTS & BUFFERS

- **RIPA buffer**

50 mM	Tris-HCl, pH 7.4	25 ml of 1M
1%	NP-40	5 ml
0.5%	Na-deoxycholate	2.5 g
0.1%	SDS	0.5 g
150 mM	NaCl	15 ml of 5M
2 mM	EDTA	2 ml of 0.5M
50 mM	NaF	1.05 g
		Final 500 ml

Store at 4˚C. Add the protease/phosphatase inhibitors (see below table)* immediately prior use.

- **Lysis 250 buffer**

50 mM	Tris-HCl, pH 7.4	25 ml of 1M
0.5 %	NP-40	2.5 ml
250 mM	NaCl	25 ml of 5M
5 mM	EDTA	5 ml of 0.5M
50 mM	NaF	1.05 g
		Final 500 ml

Store at 4˚C. Add the protease/phosphatase inhibitors (see below table)* immediately prior use.

*** Common Protease and Phosphatase Inhibitors**

Name	Stock Conc	Stock as	Working Conc
Phenylmethylsulfonyl fluoride (PMSF) 100 mM stock solution in isopropanol; store -20˚C in aliquots	100 mM	100X	1 mM
Benzamidine-HCl Store -20˚C in aliquots, 100 mM in methanol	100 mM	100X	1 mM
Leupeptin (Leu) store frozen in aliquots, 1 mg/ml in H2O	1 mg/ml	100X	0.5 µg/ml
Aprotinin (Apr) store frozen in aliquots, 1 mg/ml in H2O	1 mg/ml	100X	0.5 µg/ml
Pepstatin (Pep) store frozen in aliquots, 1 mg/ml in methanol	1 mg/ml	100X	1 mg/ml
Sodium Fluoride (NaF)	already in RIPA	---	50 mM
Sodium Orthovanadate (Na3VO4)** see the following protocol to prepare and activate Na-Orthovanadate stock	200 mM	1000X	0.2 mM

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**** Activation of Sodium Orthovanadate**

1. Sodium orthovanadate should be activated for maximal inhibition of protein phosphotyrosyl-phosphatases.
2. Prepare a 200 mM solution of sodium orthovanadate.
3. Adjust the pH to 10.0 using either 1 N NaOH or 1 N HCl. The starting pH of the sodium orthovanadate solution may vary with lots of the chemical. At pH 10.0 the solution will be yellow.
4. Boil the solution until it turns colorless (approximately 10 minutes).
5. Cool to RT.
6. Readjust the pH to 10.0 and repeat steps 3 and 4 until the solution remains colorless and the pH stabilizes at 10.0.
7. Store the activated sodium orthovanadate as aliquots at -20˚C.

This procedure depolymerizes the vanadate, converting it into a more potent inhibitor of protein tyrosine phosphatases.

METHOD

I. Monolayer Cells

1. The following steps should be performed on ice or at 4˚C using pre-cold buffers. Remove culture medium and rinse a subconfluent, 100 mm cell culture plate with PBS twice.
2. Detach cells with a rubber policeman in 1 ml cold PBS and transfer cell suspension into a 1.5 ml microcentrifuge tube.
3. Pellet cells by centrifuging at 3,000 rpm for 5 min. Remove the supernatant.
4. Suspend the pellet with 1.0 ml cold RIPA buffer (or other appropriate buffer) with freshly added (Protease Inhibitors) and/or (Phosphatase Inhibitors).
5. Allow the tube to stand on ice for 30 min, vortex every 10 min.
6. Centrifuge the resulting mixture at 14,000 x g for 15 min at 4˚C. This separates the total protein (supernatant) from the cellular debris (pellet).
7. Transfer supernatant to a new tube for further analysis.
8. The cell lysate can be frozen at this point for long-term storage at -80˚C.

II. Suspension Cells

1. Collect approximately 5.0 x 107 cells by low-speed centrifugation at RT for 5 min. Carefully remove culture medium.
2. Wash the pellet with PBS at RT, and collect by low-speed centrifugation. Carefully remove supernatant.
3. Add 1.0 ml of pre-cold RIPA buffer (or other appropriate buffer) with freshly added (Protease Inhibitors) and/or (Phosphatase Inhibitors). Gently resuspend cells in RIPA buffer with a pipet and incubate on ice for 30 min.

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4. Further disrupt and homogenize cells by passing through a 21-gauge needle, dounce homogenization or sonication, taking care not to raise the temperature of the lysate. (Optional: Add 10 µl of 10 mg/ml PMSF stock) Incubate 30 min on ice.

5. Transfer to microcentrifuge tube(s) and centrifuge at 10,000 x g for 10 min at 4˚C. The supernatant fluid is the total cell lysate. Transfer the supernatant to a new microfuge tube and discard the pellet.

III. Tissue Samples

1. Weigh tissue and dice into very small pieces using a clean razor blade. Frozen tissue can be sliced very thinly and thawed in RIPA buffer containing (Protease Inhibitors) and/or (Phosphatase Inhibitors). Use 3ml of pre-cold RIPA buffer per gram of tissue.

2. Further disrupt and homogenize tissue with a dounce homogenizer or a sonicator, while maintaining temperatures at 4˚C throughout all procedures. (Optional: Add 30 µl of 10 mg/ml PMSF stock per gram of tissue.) Incubate on ice for 30 min.

3. Transfer to microcentrifuge tubes, centrifuge at 10,000 x g for 10 min at 4˚C. Remove supernatant and centrifuge again. The supernatant fluid is the total cell lysate. A longer centrifugation may be necessary to obtain a clear lysate.

ABOUT

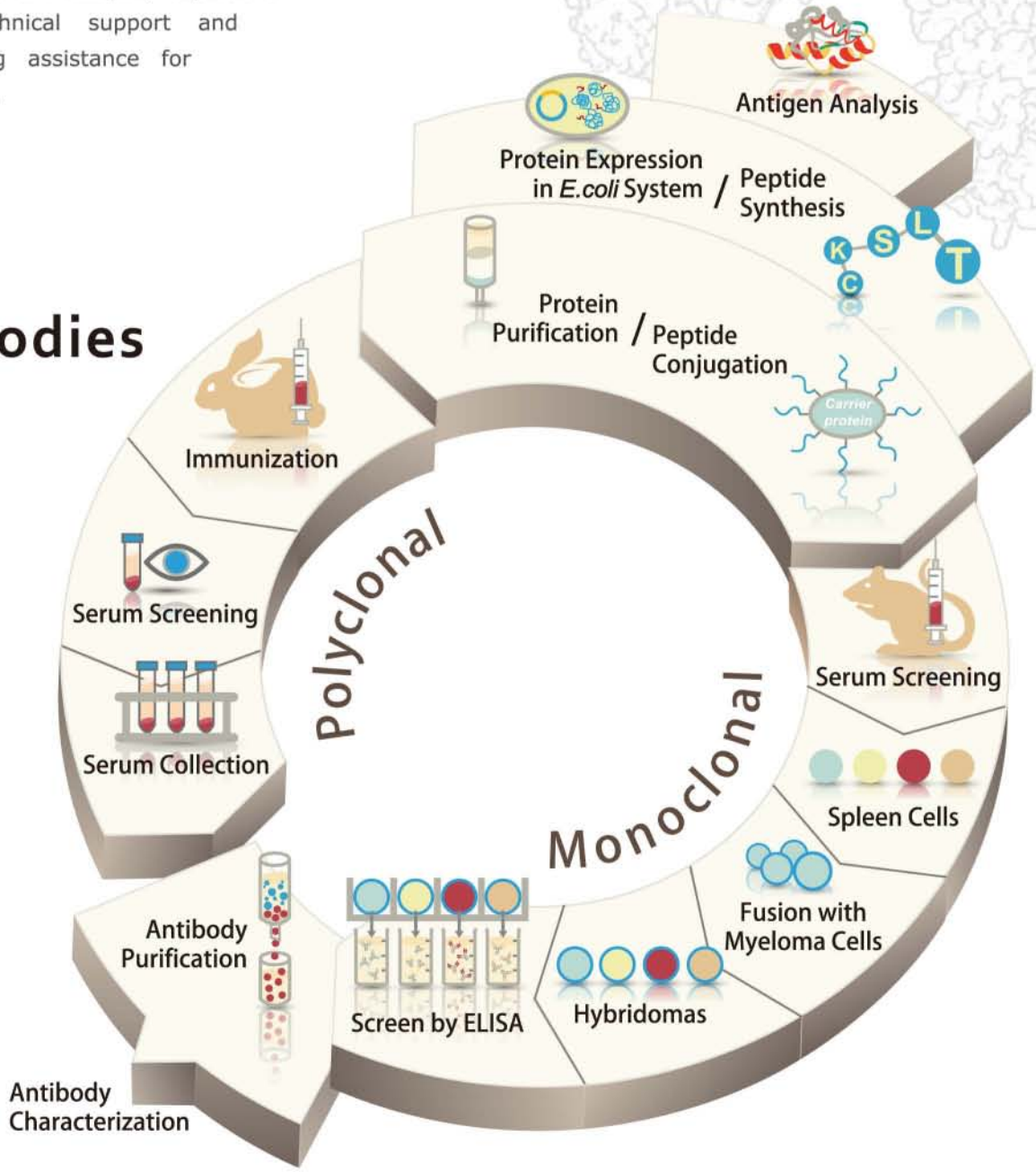
GeneTex

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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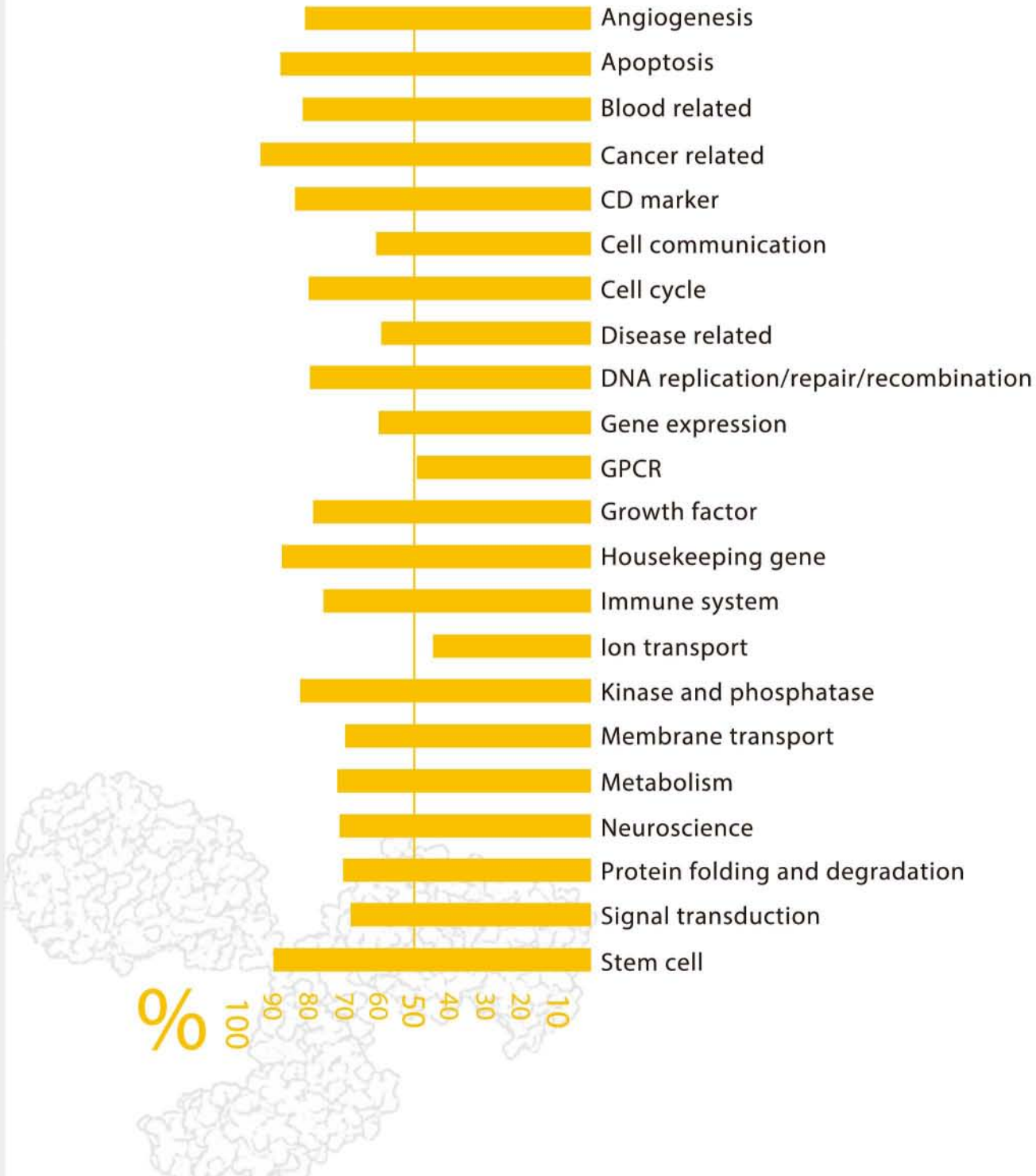

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