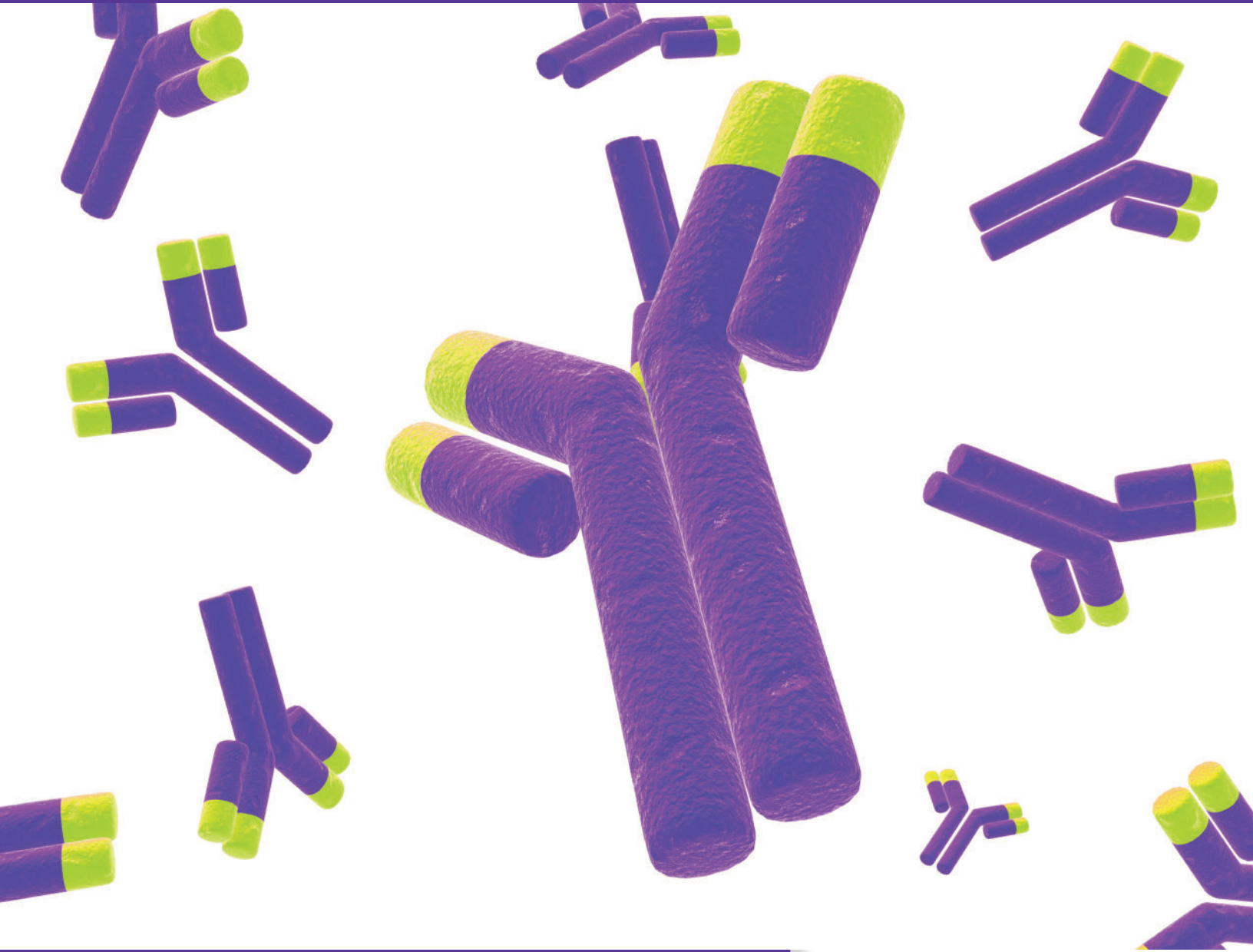


SouthernBiotech Custom Services



Quality Antibodies for Quality Research

SouthernBiotech

www.SouthernBiotech.com info@SouthernBiotech.com



Custom Services

Peptide Synthesis for Antibody Production

SouthernBiotech provides complete services for production of immunogenic peptides for antibody development. These services include -

Fmoc chemistry available for peptide synthesis

Synthesis of multiple antigenic peptides (MAPs)

Purification of synthesized peptide to achieve a purity of 95% (other levels of purity available upon request)

Evaluation of synthesized peptide by mass spectrometry, amino acid sequence analysis, and HPLC methods to ensure sequence integrity and purity prior to conjugation

Consultation available to select the appropriate chemistry for conjugation of peptide to carrier proteins

Conjugation to carrier protein and purification of peptide/carrier complex by gel filtration chromatography

Available Services for Peptide Synthesis

| | Cat. No. |
|------------------------------------------------------------------|----------|
| Peptide Synthesis (includes mass spectrometry and HPLC analysis) | SBCS-20 |
| Peptide Purification (95% purity by mass spectrometry) | SBCS-21 |

Polyclonal Antibody Development and Production Services

Available Services for Polyclonal Antibody Development and Production

| | Cat. No. |
|--------------------------------------------------------------|----------|
| Conjugation to KLH for Immunization | SBCS-22K |
| Conjugation to BSA for Evaluating Antibody Titer | SBCS-22B |
| Homogenization of Antigen from Gels or Nitrocellulose Slices | SBCS-19 |
| ELISA testing of Antiserum | SBCS-60 |

Rabbit Production Services - Standard Ninety (90) Day Protocol

| | |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Exclusive use of animals, housing, maintenance, pre-immune bleed, and Six (6) injections | SBCS-23 |
| Day Thirty (30) - First Bleed | SBCS-23I |
| Day Sixty (60) - Second Bleed | SBCS-23II |
| Day Ninety (90) - Third Bleed - Serum shipped for evaluation | SBCS-23III |
| Project Termination | SBCS-23T |
| Note - All animals will be terminated Ten (10) days after the last scheduled shipment unless we receive written instructions by Day 100 to continue maintaining the animals for a specified period at our per diem rate per rabbit | |
| Per Diem per Rabbit for Project Extension | SBCS-RC |

Note - It is recommended that multiple animals are used in order to maximize potential of immune response

Polyclonal Antibody Development and Production Services

| Available Services Polyclonal Antibody Development and Production | Cat. No. |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|
| Goat/Sheep Production Services - Standard Ninety (90) Day Protocol | |
| Exclusive use of animals, housing, maintenance, pre-immune bleed, and Six (6) injections | SBCS-24 |
| Day Thirty (30) - First Bleed | SBCS-24I |
| Day Sixty (60) - Second Bleed | SBCS-24II |
| Day Ninety (90) - Third Bleed - Serum shipped for evaluation | SBCS-24III |
| Project Termination | SBCS-24T |
| Note - All animals will be terminated Ten (10) days after the last scheduled shipment unless we receive written instructions by Day 100 to continue maintaining the animals for a specified period at our per diem rate per goat/sheep | |
| Per Diem per Goat/Sheep for Project Extension | SBCS-GC |
| Donkey Production Services - Standard Ninety (90) Day Protocol | |
| Exclusive use of animals, housing, maintenance, pre-immune bleed, and Six (6) injections | SBCS-25 |
| Day Thirty (30) - First Bleed | SBCS-25I |
| Day Sixty (60) - Second Bleed | SBCS-25II |
| Day Ninety (90) - Third Bleed - Serum shipped for evaluation | SBCS-25III |
| Project Termination | SBCS-25T |
| Note - All animals will be terminated Ten (10) days after the last scheduled shipment unless we receive written instructions by Day 100 to continue maintaining the animals for a specified period at our per diem rate per donkey | |
| Per Diem per Donkey for Project Extension | SBCS-DC |

Custom Services

Hybridoma Development

SouthernBiotech offers services for the development of monoclonal antibodies. Our Hybridoma Development Services include - immunization, cell fusion, cloning, and characterization of your monoclonal antibody.

| Available Services for Hybridoma Development | Cat. No. |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|
| Pre-Immunization Antigen Processing and Modification | |
| Homogenization of Antigen in Gels or Nitrocellulose Slices | SBCS-19 |
| Conjugation to KLH for Immunization | SBCS-22K |
| Conjugation to BSA for Screening | SBCS-22B |
| Dialysis into Physiological Buffer | SBCS-22D |
| Phase I - Immunization and Evaluation of Antibody Titer | SBCS-30I |
| Immunization of Three (3) BALB/c mice with your supplied and purified antigen. Includes Four (4) immunizations per our standard protocol and daily care. Test bleeds and ELISA data will be provided for your evaluation and decision to continue development. | |
| Phase II - Fusion, Plating, and Screening | SBCS-30II |
| Splenocytes of one of the mice are fused with a selected myeloma cell line. All specific antibody producing colonies are selected by ELISA for expansion. Supernatant from Five (5) of the best hybridoma-containing wells will be shipped to you for screening. Cryopreservation of up to Five (5) positive clones. | |
| Phase III - Cloning, Expansion, and Cryopreservation | SBCS-30III |
| Subcloning of one positive clone to monoclonality by two rounds of limiting dilution. ELISA screening to detect positive antibody-secreting subclones. Cryopreservation of Ten (10) vials of the hybridoma cell line and isotyping of one monoclonal antibody. | |
| Phase IV - Amplification | SBCS-30IV |
| Production and shipment of monoclonal antibody supernatant from one selected clone | |

Monoclonal Antibody Services

SouthernBiotech offers a broad range of services for the production of monoclonal antibodies. Our Monoclonal Antibody Production Services include production of your monoclonal antibody *in vivo* in BALB/c or immunocompromized (e.g., SCID, Nu/Nu, RAG-/-) mice, or *in vitro* in flasks or bioreactors. Additional services include antibody purification and conjugation to fluorochromes and enzymes.

| Available Services for Monoclonal Production | Cat. No. |
|---------------------------------------------------------------------------------------|-----------------|
| Subcloning, Expansion, and Screening of Clones | SBCS-30S |
| Cryopreservation - Up to Twenty (20) Clones per Month | SBCS-30C |
| Mycoplasma Testing | SBCS-29 |
| Ascites Production in Ten (10) BALB/c Mice | SBCS-31 |
| Ascites Production in Twenty (20) BALB/c Mice | SBCS-32 |
| Ascites Production in Fifty (50) BALB/c Mice | SBCS-33 |
| Ascites Production in One-Hundred (100) BALB/c Mice | SBCS-34 |
| Ascites Production in Ten (10) Immunocompromised Mice | SBCS-35 |
| Ascites Production in Twenty (20) Immunocompromised Mice | SBCS-36 |
| Ascites Production in Fifty (50) Immunocompromised Mice | SBCS-37 |
| Ascites Production in One-Hundred (100) Immunocompromised Mice | SBCS-38 |
| <i>In vitro</i> Production in Cell Culture Flask - Fifty (50) to One-Hundred (100) mL | SBCS-91 |
| <i>In vitro</i> Production in Cell Culture Flask - Thirty (30) mL | SBCS-92 |
| <i>In vitro</i> Production in Bioreactor | SBCS-90 |

Note - Before initiating an antibody development project, the customer must provide SouthernBiotech with a signed assurance that any customer-supplied material/antigen is mycoplasma-free, non-infectious, non-carcinogenic, and has no known safety concerns to animals or humans.

Custom Services

Purification Services

SouthernBiotech uses a variety of methods for purification of antibody from serum, ascites, and culture supernatants including $(\text{NH}_4)_2\text{SO}_4$ salt precipitation, ion exchange and size exclusion chromatography, Protein A and Protein G affinity chromatography, and custom affinity chromatography. The method employed is dictated by the species, the source material, and the level of immunoglobulin purity required. The amount of supernatant or serum required per column depends on the concentration and total milligrams of the solution.

| Available Services for Protein Purification | Cat. No. |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|
| Affinity Purification Antibodies of extremely high purity and specificity can be obtained using custom affinity chromatography. A minimum of 10 mg of pure antigen (or purified peptide) is needed to perform this procedure. | <u>SBCS-45</u> |
| Ammonium Sulfate Precipitation Ammonium sulfate is widely used in isolating both IgM and IgG from a variety of species. A gel filtration and/or an ion exchange step can be employed to further purify the immunoglobulin of interest. | <u>SBCS-40</u> |
| Ion Exchange Chromatography This method is useful as an adjunct to ammonium sulfate precipitation for purifying antibodies from ascites fluid or where Protein A and Protein G elution conditions may damage the antibody. It can also be used for removing trace quantities of DNA contaminants. | <u>SBCS-41</u> |
| Protein A Purification High yields of pure IgG can be obtained in a single-step purification using Protein A affinity chromatography, which differ in their ability to bind antibodies of different origins and IgG subclasses. | <u>SBCS-43</u> |
| Protein G Purification High yields of pure IgG can be obtained in a single-step purification using Protein G affinity chromatography, which differ in their ability to bind antibodies of different origins and IgG subclasses. | <u>SBCS-44</u> |
| Size Exclusion/Gel Filtration Chromatography A single gel matrix or a sequence of size exclusion columns can be used for purification of IgM and IgG antibodies. The purification strategy employed is dependent on the immunoglobulin class and the source of the material. | <u>SBCS-42</u> |
| Low Endotoxin Purification Purification under aseptic conditions to generate clean, low-endotoxin antibody preparations. | <u>SBCS-48</u> |
| Purification of IgY from Chicken Egg Yolks Since IgY is not bound by typical IgG affinity resins (Protein G or Protein A), we use proprietary methods to deliver highly pure IgY. | <u>SBCS-47</u> |

Fragmentation Services

Antibody fragmentation services for 5 - 20 mg of antibody includes dialysis, digestion, concentration, and purification of the fragments.

| Available Services for Antibody Fragmentation | Typical Yield | Cat. No. |
|------------------------------------------------------|----------------------|-----------------|
| Fc Fragmentation | 15 - 20% | <u>SBCS-71</u> |
| Fab Fragmentation | 15 - 20% | <u>SBCS-72</u> |
| F(ab) ₂ Fragmentation | 30 - 40% | <u>SBCS-73</u> |

Conjugation Services

| Available Services for Antibody Conjugation | Cat. No. |
|-------------------------------------------------------|----------|
| Alexa Fluor® 488 (AF488) 2 - 10 mg | SBCS-88 |
| Alexa Fluor® 555 (AF555) 2 - 10 mg | SBCS-99 |
| Alexa Fluor® 647 (AF647) 2 - 10 mg | SBCS-89 |
| Alexa Fluor® 700 (AF700) 2 - 10 mg | SBCS-98 |
| Alkaline Phosphatase (AP) 2 - 10 mg | SBCS-57 |
| Allophycocyanin (APC) 2 - 10 mg | SBCS-55 |
| Allophycocyanin/Cyanine 5.5 (APC/CY5.5) 2 - 10 mg | SBCS-77 |
| Allophycocyanin/Cyanine 7 (APC/CY7) 2 - 10 mg | SBCS-78 |
| β-galactosidase (BGAL) 2 - 20 mg | SBCS-49 |
| Biotin (BIOT) 2 - 20 mg | SBCS-59 |
| Cyanine 3 (CY3) 2 - 10 mg | SBCS-54A |
| Cyanine 5 (CY5) 2 - 10 mg | SBCS-54B |
| Fluorescein Isothiocyanate (FITC) 2 - 20 mg | SBCS-50 |
| Horseradish peroxidase (HRP) 2 - 20 mg | SBCS-58 |
| Pacific Blue™ (PACBLU) 2 - 10 mg | SBCS-97 |
| R-Phycoerythrin (PE) 2 - 10 mg | SBCS-53 |
| R-Phycoerythrin/Cyanine 5.5 (PE/CY5.5) 2 - 10 mg | SBCS-75 |
| R-Phycoerythrin/Cyanine 7 (PE/CY7) 2 - 10 mg | SBCS-76 |
| R-Phycoerythrin/Texas Red® (PE/TXRD) 2 - 10 mg | SBCS-74 |
| Sepharose® (SEPH) 2 - 10 mg | SBCS-69 |
| Spectral Red® (SPRD) 2 - 10 mg | SBCS-56 |
| Tetramethylrhodamine Isothiocyanate (TRITC) 2 - 20 mg | SBCS-51 |
| Texas Red® (TXRD) 2 - 20 mg | SBCS-52 |

Note - Antibodies should be supplied at a minimum concentration of 5 mg/mL in neutral buffer without preservatives or protease inhibitors. Other custom conjugates available upon request. Conjugated antibodies can be tested in an appropriate immunoassay upon customer request.

Additional Services

| Available Services | Cat. No. |
|-------------------------|----------|
| Antibody Concentration | SBCS-22C |
| Antibody Lyophilization | SBCS-22L |

Custom Services

Characterization/Testing Services

SouthernBiotech employs several techniques to evaluate the quantity, quality, and function of purified antibodies, including - enzyme linked immunosorbent assay (ELISA), flow cytometry, immunoblotting, SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Limulus Amoebocyte Lysate (LAL) assay.

| Available Services for Protein Characterization/Testing | Cat. No. |
|----------------------------------------------------------------|-----------------|
| ELISA Testing for Antigen-Specific Activity | SBCS-60 |
| ELISA Testing for Isotype Determination | SBCS-61 |
| Endotoxin Testing by LAL Assay | SBCS-64 |
| Flow Cytometry Analysis | SBCS-65 |
| HPLC | SBCS-66 |
| Immunoblotting | SBCS-63 |
| SDS-PAGE Analysis | SBCS-62 |
| Biotin Quantitation by HABA/Avidin | SBCS-67 |

To initiate a Custom Service Project contact our Technical Services Department at 205.945.1774 or technical@southernbiotech.com. A project manager will be assigned and report to you regularly on an agreed schedule. Discounts and third party arrangements are not accepted in conjunction with SouthernBiotech Custom Services.

Corporate Offices

160 Oxmoor Blvd
Birmingham, AL 35209
USA

Mailing Address

PO Box 26221
Birmingham, AL 35260
USA

Tel 205.945.1774

Fax 205.945.8768

Email info@SouthernBiotech.com

Technical Support technical@SouthernBiotech.com

Website www.SouthernBiotech.com

SouthernBiotech is ISO 9001:2015 Certified

SouthernBiotech

www.SouthernBiotech.com info@SouthernBiotech.com



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