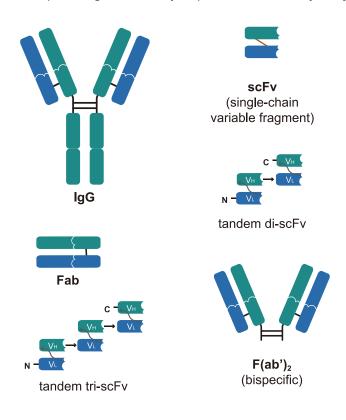


### Recombinant Antibody Production

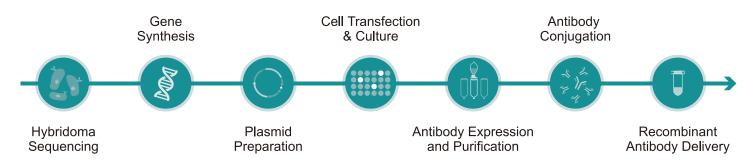
The production of engineered antibodies has helped advancing the progress of research projects and drug development. Synbio Technologies has continued to serve this progression by offering our clients recombinant antibody preparation services, including full-length antibodies (IgG), single chain antibodies (scFv), antigen binding fragments (Fab), and bispecific antibodies. Our comprehensive antibody engineering solutions take your projects from hybridoma sequencing to antibody expression, antibody conjugationin as few as 2-3 weeks.



# Why Choose Synbio Technologies?

- Our experienced team has successfully delivered more than 10,000 projects.
- ★ We offer a full suite of solutions, saving you time and money on outsourcing needs.
- Our proven processes and proprietary systems allow us to deliver your projects in as few as
   2-3 weeks.
- Our quality speaks for itself. We have delivered thousands of projects with a >95% success rate.

#### **Service Procedure**



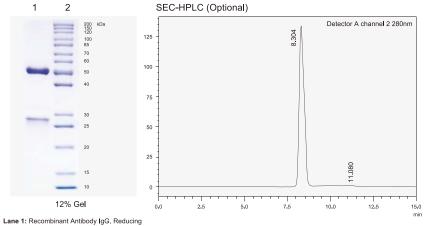
#### **Service Specifications**

Projects	Service Details	Deliverables
Antibody Discovery	Hybridoma sequencing or immune repertoire sequencing (optional)	Antibody gene sequencing report
Gene Synthesis	<ul> <li>Gene synthesis and vector construction: IgG, Fab, scFv, VHH, BsAb, etc.</li> <li>Plasmid Preparation</li> </ul>	2-5 μg lyophilized plasmids
Recombinant Antibody Expression and Purification	<ul> <li>Cell transfection &amp; culture</li> <li>30 ml, 100 ml, 200 ml or larger scale recombinant antibody expression</li> <li>Antibody affinity purification and purity detection (SDS-PAGE, SEC-HPLC), purity&gt; 95%</li> <li>Endotoxin: &lt;1 EU/mg</li> </ul>	Purified antibody     QC report
Antibody Conjugation	<ul> <li>Conjugation with fluorescent dyes, enzymes, or toxins</li> <li>Common formats: HRP, FITC, PE, APC, biotin, drug payloads</li> <li>Optimization of conjugation ratio for activity retention</li> <li>QC by SDS-PAGE, and functional assay</li> </ul>	Conjugated antibody     Conjugation QC     report

## Case Study 1 >95% Purity IgG with <1 EU/mg Endotoxin

Obtaining accurate expression and high-purity antibodies directly impacts the results of subsequent experiments. Clients only need to provide gene sequences, such as IgG, and the >95% purity is detected by SDS-PAGE. For accurate detection, the SEC-HPLC detection method can be used, which can be up to 99.5% correct. Endotoxin level is at minimum standard, < 1 EU/mg, which allows clients to achieve high efficacy in subsequent experiments.

#### **SDS-PAGE**



Detector A Channel 2 280nm

Peak#	Ret.Time	Area	Height	Conc.
1	8.304	2782111	124129	99.524
2	11.080	2427374	722	0.476
Total		2795426	124850	

QC Results					
Test Items	Assay Description	Results			
Purity	SDS-PAGE	>95%			
Purity (Optional)	SEC-HPLC	99.524%			
Endotoxin	LAL method	<1 EU/mg			

Lane 2: Protein Marker

### Case Study 2 FITC conjugated antibody

- Starting material: polyclonal antibody
- Fluorescent dye: FITC (Fluorescein Isothiocyanate)
- <u>Method:</u> the immune serums are purified by affinity chromatography to obtain high-purity IgG. Using direct labeling method, the FITC is conjugated to the IgG antibody molecule on the lysine group.

#### QC test result:

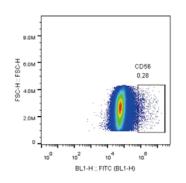
	A280	A495	F/P*
FITC conjugated Ab	1.523	1.761	5.6

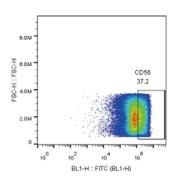
<sup>\*</sup> F/P values of 2.5-6.5 are probably optimal for any partciular IgG. F/P=3.1×A495/[A280-0.31×A495]

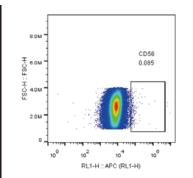
Antibody FITC labeling yield (80%) and conjugation efficiency (F/P=5.6) are high.

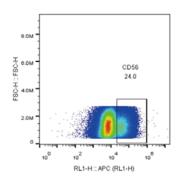
### Case Study 3 FITC & APC-cy7 Conjugated anti-CD56 Antibody

- Starting material: FITC anti-CD56 antibody, APC-cy7 anti-CD56 antibody
- Fluorescent dye: FITC, APC-cy7 (APC-Cyanine 7)
- Test materials: HEK-293 transient transfection cells and negative control cells
- <u>Method</u>: CD56 (FITC & APC-cy7 conjugated) antibodies are tested in FACS experiments using cultured HEK-293 cells expressing recombinant CD56 proteins and negative control cells.









HEK-293 negative control cells

CD56 transfection cells

HEK-293 negative control cells

CD56 transfection cells

Staining with anti-CD56 FITC

Staining with anti-CD56 APC-cy7

CD56 (FITC & APC-cy7 conjugated) antibodies are suitable for FACS application.

#### Case Study 4 FITC Conjugated anti-CD25 Antibody

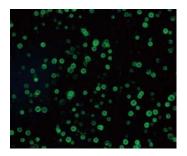
- Starting material: FITC anti-CD25 antibody
- Fluorescent dye: FITC (Fluorescein Isothiocyanate)
- Test materials: HEK-293 transient transfection cells and negative control cells
- Method: CD25 (FITC conjugated) antibody is tested in immunofluorescence experiments using cultured HEK-293 cells expressing recombinant CD25 proteins and negative control cells.

# Staining with anti-CD25 FITC HEK-293 negative control cells (light field)

Staining with anti-CD25 FITC



CD25 transfection cells (light field)



CD25 transfection cells (dark field)

CD25 (FITC conjugated) antibody is utilized to mark cell surface antigens even at low concentration.





HEK-293 negative control cells

(dark field)

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