

技术与方法

### 抗抗CD3 ScFv单克隆抗体的制备和活性检测

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**摘要** 摘要: 目的 制备抗抗CD3 ScFv单克隆抗体,用以抗CD3抗体的亲和层析纯化及血清中该类抗体浓度的检测。方法 采用常规免疫学方法制备抗抗CD3 ScFv单克隆抗体并制备抗抗CD3 ScFv单克隆抗体免疫亲和层析柱,用于抗CD3 ScFv蛋白和去除E-tag的Diabody [CD3×Pgp]的分离纯化。采用FACS法测定分别经抗抗CD3 ScFv抗体免疫亲和层析柱及抗E-tag亲和层析柱纯化的抗CD3 ScFv蛋白和Diabody [CD3×Pgp]对K562/A02和Jurkat细胞特异结合活性。采用间接ELISA法进行抗抗CD3 ScFv抗体特异性结合活性的检测。结果 抗抗CD3 ScFv单克隆抗体能特异性地与抗CD3 ScFv蛋白结合而不与血清发生反应。经抗抗CD3 ScFv抗体免疫亲和层析柱和经抗E-tag亲和层析柱纯化后的抗CD3 ScFv蛋白均能与Jurkat细胞特异结合。经抗抗CD3 ScFv抗体免疫亲和层析柱纯化的去除E-tag的Diabody [CD3×Pgp]与K562/A02和Jurkat细胞结合的阳性率分别为89.87%和83.95%;与其亲代抗体竞争结合K562/A02和Jurkat细胞后,结合率分别下降为56.30%和43.78%。结论 制备了抗抗CD3单克隆抗体和亲和层析柱,可用于抗CD3抗体的亲和层析纯化及血清中该类抗体浓度的检测。

**关键词** [抗CD3 ScFv蛋白](#) [单克隆抗体](#) [免疫亲和层析](#) [Diabody \[CD3×Pgp\]](#)

分类号

### Preparation and Activity Detection of Monoclonal Antibody against Anti-CD3 ScFv

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**Abstract** ABSTRACT: Objective To prepare monoclonal antibody (McAb) against anti-CD3 ScFv for purifying and detecting serum anti-CD3 antibody concentration. Methods McAb against anti-CD3 ScFv was prepared by hybridoma technique and used to prepare affinity chromatography column, which was used to purify anti-CD3 ScFv and Diabody [CD3×Pgp] without E-tag. The binding activities of anti-CD3 ScFv, Diabody [CD3×Pgp] without E-tag, and Diabody [CD3×Pgp] purified by anti-CD3 affinity chromatography column or anti-E-tag affinity chromatography column against K562/A02 cell and Jurkat cells were detected by fluorescence activated cell sorting (FACS) method. ELISA was used to identify the specificity of the McAb. Results McAb against anti-CD3 ScFv specifically detected serum anti-CD3 ScFv without reacting with sera. The anti-CD3 ScFv purified by anti-CD3 affinity chromatography column and purified by anti-E-tag affinity chromatography column had the same specific binding activity with Jurkat cells. The positive binding rates of Diabody [CD3×Pgp] without E-tag to K562/A02 and Jurkat cells were 89.87% and 83.95%, respectively. In the competitive binding experiments with K562/A02 and Jurkat cells, the binding rates of Diabody [CD3×Pgp] without E-tag decreased to 56.30% and 43.78%, respectively. Conclusion The McAb against anti-CD3 ScFv prepared in our lab can be used to purify and detect serum anti-CD3 antibody concentration.

**Key words** [anti-CD3 ScFv](#) [monoclonal antibody](#) [affinity chromatography](#) [Diabody \[CD3×Pgp\]](#)

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