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efficiency.

#### 5. Purify the conjugation

The following protocol is an example of dye-protein conjugate purification by using a SepHAdex G-25 column.

- 1) Prepare SepHAdex G-25 column according to the manufacture instruction.
- 2) Load the reaction mixture (From "Run conjugation reaction") to the top of the SepHAdex G-25 column.
- 3) Add PBS (pH 7.2-7.4) as soon as the sample runs just below the top resin surface.
- 4) Add more PBS (pH 7.2-7.4) to the desired sample to complete the column purification. Combine the fractions that contain the desired dye-protein conjugate.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

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## REFERENCES

- [1]. Ptaszek M. Rational design of fluorophores for in vivo applications. *Prog Mol Biol Transl Sci.* 2013;113:59-108.
- [2]. Shindy, H. A. (2017). Fundamentals in the chemistry of cyanine dyes: A review. *Dyes and Pigments*, 145, 505–513.
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**Caution: Product has not been fully validated for medical applications. For research use only.**

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