

Supplementary Information

Quantitative Brain-Derived Neurotrophic Factor Lateral Flow Assay for Point-of-Care Detection of Glaucoma

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Table S1 Summary of studies to investigate the role of BDNF on glaucoma

Study	Subjects	Treatment	Results	Conclusion
Pease et al ⁷	Rat acute IOP elevation model	Autoradiography of ¹²⁵ I-BDNF	The eyes with IOP elevation had a 60% reduction in transported radioactivity overlying the inner retina compared to the mean of control eyes.	BDNF retrograde transport was interrupted in glaucoma animal models.
	Juvenile cynomolgus monkeys' optic nerve axotomy model	Immunohistochemistry label of BDNF	There was a significant decrease in axonal label for BDNF. After 7 days of treatment, the staining of BDNF was decreased from the nerve head and superior retina	Injury of RGCs leads to the decrease of BDNF.
Johnson et al ⁸	Rat hypertonic saline model	Immunohistochemistry label of BDNF	BDNF delayed the RGC death to 5 days. After 14 days of axotomy, 45% RGCs had survived in BDNF inject group but only 18% in control group.	Exogenous BDNF was able to protect the RGCs.
Galindo-Romero et al ⁹	Mouse axotomy model	Intravitreal injection of BDNF		
Oddone et al ¹⁰	45 patients with glaucoma at different stage. 15 healthy controls	Detection of serum level of BDNF	Glaucoma: 261.2±75.0 pg·mL ⁻¹ Healthy subjects: 313.6±79.6	Serum level of BDNF significantly decreased in glaucoma patients,

			pg·mL ⁻¹ , p = 0.03	especially in early and moderate stages.
Igarashi et al ¹¹	27 patients with glaucoma and 51 healthy control subjects	Detection of serum level of BDNF	Glaucoma: 7.2 ± 3.6 ng·mL ⁻¹ Healthy subjects: 12.2 ± 9.3 ng·mL ⁻¹ , p=0.004	Serum BDNF concentration was lower in glaucoma patients than in healthy people in Japan.
Ghaffariyeh et al ¹²	25 patients with glaucoma and 25 healthy control subjects	Detection of serum level of BDNF	Glaucoma: 18.42 ± 4.05 ng·mL ⁻¹ Healthy subjects: 27.16 ± 5.53 ng·mL ⁻¹ , p< 0.05	Serum level of BDNF significantly decrease in glaucoma group.
		Detection of tear level of BDNF	Glaucoma: 78.0 ± 25.1 pg·mL ⁻¹ Healthy subjects: 116.2 ± 43.1 pg·mL ⁻¹ , p< 0.001	
Shpak et al ¹³	55 patients with POAG and 29 healthy control subjects	Detection of aqueous humor level of BDNF	Glaucoma: 35.2 ± 14.2 pg·mL ⁻¹ Healthy subjects: 54.6 ± 29.6 pg·mL ⁻¹ , p< 0.001	BDNF contents are significantly decreased in aqueous humor, tear and blood serum in patients with POAG.
		Detection of serum level of BDNF	Glaucoma: 19230 ± 5960 pg·mL ⁻¹ Healthy subjects: 22440 ± 7580 pg·mL ⁻¹ , p< 0.02	

Table S2 Comparison between commercial ELISA and LFA strip

	ELISA	LFA
Response time	90 mins	30-40 mins
Detection limit	12.83 pg·mL ⁻¹ (15.6 pg·mL ⁻¹)*	14.12 pg·mL ⁻¹
Intra-assay CV%	2.8%	7.6%
Inter-assay CV%	5.3%	9.0%
Recovery	85% - 112%	88.76%-103.87%
Selectivity	High	High
pH affects	Low	Low

CV: Coefficient of Variation; *According to the abcam, the detect limit of the Human BDNF ELISA kit is 15.6 pg·mL⁻¹

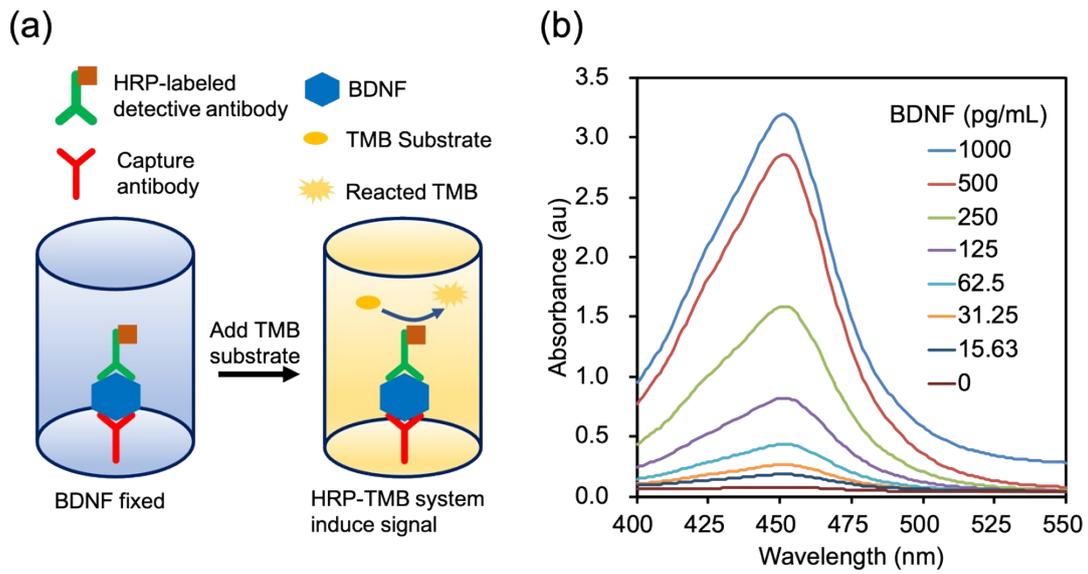


Figure S1 Schematic illustration of ELISA mechanism and the absorbance spectrum.

a) The schematic figure indicates the mechanism of conventional ELISA kit. b) The spectrum reading of each group.

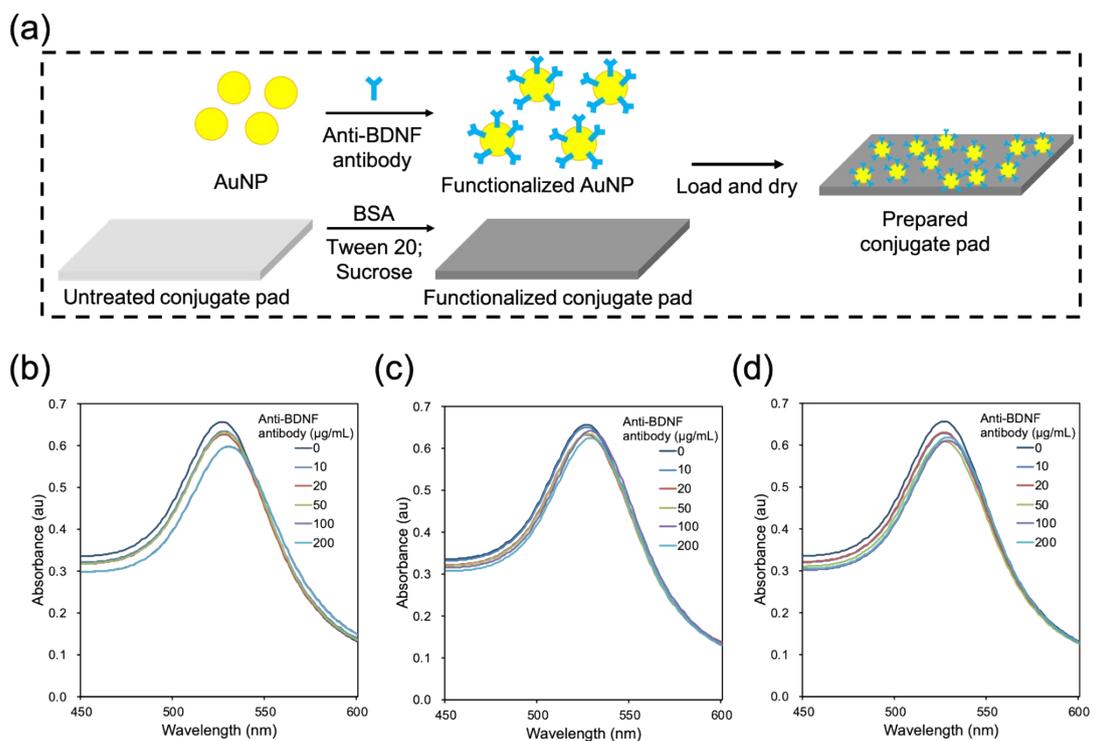


Figure S2 Schematic illustration of the fabrication of conjugate pad and optimization of pH and antibody volume for conjugation. a) Schematic illustration of the fabrication of conjugate pad. The antibody was physically absorbed at the surface of the AuNP which then increased the size of the conjugate. The conjugate pad was pre-treated with a buffer that contained BSA, Tween 20, and sucrose. The functionalized AuNP-antibody conjugate was then loaded at the conjugate pad and dried for 1 hour at 37°C. b) The absorbance spectrum of the resulting antibody-AuNP conjugation solution under pH 8.0. c) The absorbance spectrum of the resulting antibody-AuNP conjugation solution under pH 8.5. d) The absorbance spectrum of the resulting antibody-AuNP conjugation solution under pH 9.0.