FlyLink™ Labeling Kit

Cat #: D-AKE520/D-AKE530/D-AKE540/D-AKE560/D-AKE580

Size: 100 μg*3

Storage: Stored at -20°C for 6 months, protected from light

Product information

Cat	Dye name	Maximum Excitation/ Emission Light (nm)	Extinction Coefficient (ε)	Molecular Weight (Mwt Da)
D-AKE520	FlyLink™ 488	490/515	70,000	~834
D-AKE530	FlyLink™ 555	555/565	150,000	~1077
D-AKE540	FlyLink™ 594	593/614	115,000	~927
D-AKE560	FlyLink™ 647	650/665	240,000	~1258
D-AKE580	FlyLink™ 680	680/701	140,000	~1111

Assay Principle

FlyLink™ dyes are a series of highly water-soluble fluorescent dyes spanning the visible and near-infrared spectrum for labeling biomolecules, especially proteins and nucleic acids. FlyLink™ dyes bear the core structures of different fluorescent dye that cover the fluorescence spectrum from UV to NIR. Innovative modifications to the core structure make FlyLink™ dyes superior to other commercial dyes with many innovatively novel features. Our kit has been pre-activated, and can be directly used for conjugation. **FlyLink™ Labeling Kit is designed for preparing FlyLink™ conjugates directly from proteins, peptides, and other ligands that contain a free amino group.**

FlyLink™ 488： FlyLink™ 488 is a green fluorescent dye optimally excitable by the 488 nm argon laser line, which is super alternative to FITC, Alexa Fluor 488. It yields biologically more specific conjugates and less background.

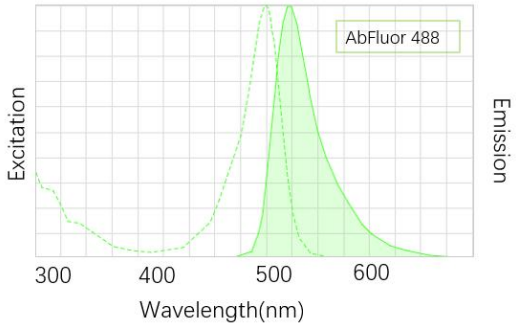

Fig.1: FlyLink™ 488 spectrogram

FlyLink™ 555: FlyLink™ 555 is a bright and photostable fluorescent dye optimally excitable by the 555 nm argon laser line, which is super alternative to TRITC, Cy3 and Alexa Fluor 555. It yields biologically more specific conjugates and less background.

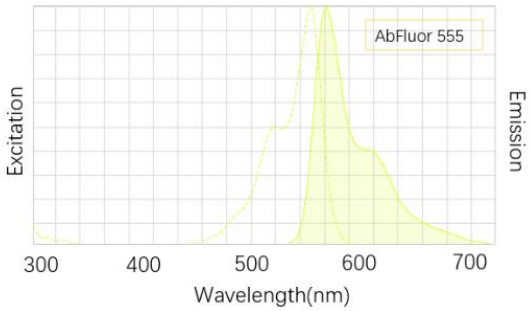

Fig.2: FlyLink™ 555 spectrogram

FlyLink™ 594: FlyLink™ 594 is the brightest deep red dye optimally excitable by the 594 nm argon laser line, which is extremely photostable and super alternative to Texas Red, DyLight 594 and Alexa Fluor 594. It yields the brightest conjugates among spectrally similar dyes.

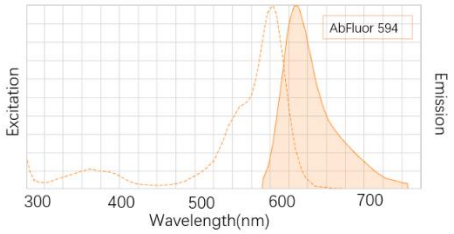

Fig.3: FlyLink™ 594 spectrogram

FlyLink™ 647: FlyLink™ 647 is a far-red fluorescent dye optimally excitable by the 647 nm argon laser line, which is super alternative to Cy5,DyLight 649 and Alexa Fluor 647. It yields biologically more specific conjugates and less background.

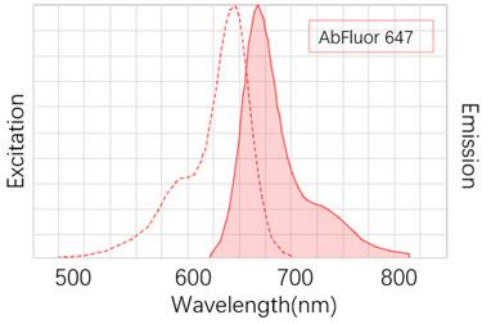

Fig.4: FlyLink™ 647 spectrogram

FlyLink™ 680: FlyLink™ 680 is an outstanding 680 nm-excitable dye, which is super alternative to Cy5.5, Dylight 680 and Alexa Fluor 680. It is the brightest among spectrally similar 680 nm dyes. It yields biologically more specific conjugates and less background.

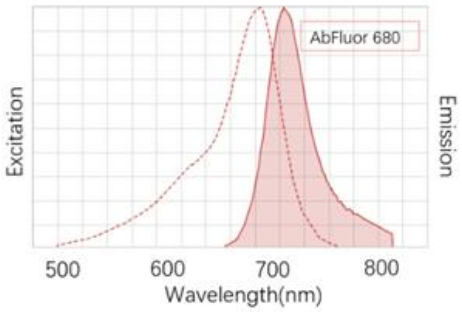

Fig.5: FlyLink™ 680 spectrogram

Application example: The coupled direct-labeled primary antibody does not require a secondary antibody in the immunoassay, thus eliminating tedious incubation and washing steps, saving time.

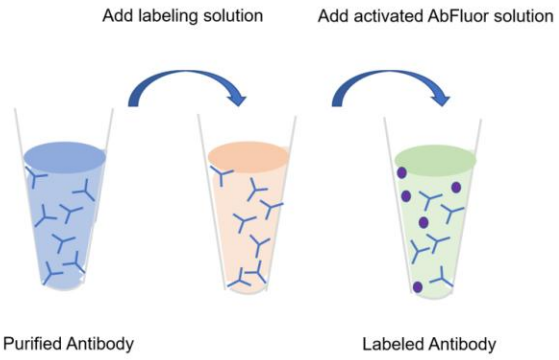

Fig.6: Schematic diagram of FlyLink™ Labeling Kit

Materials Supplied and Storage Conditions

Kit components	Size（100T）	Storage conditions
Activated FlyLink™ 488 solution	7.5 µL	-20°C, protected from light
FlyLink™ 488 labeling solution	15 µL	-20°C
Purification column (0.5 mL, 50 KD)	3	RT
Booklet	1	RT

Note: The purified column is suitable for samples with molecular weights ranging from 100 KD to 200 KD; If there are unused reagents left, please seal them and store them at -20°C in time.

Materials Required but Not Supplied

·Prepared samples to be labelled

·Precision pipettes, disposable pipette tips

·Deionized water, PBS (pH 7.4)

Sample Preparation

The initial concentration of the sample to be labeled should be higher than 2 mg/mL. It is suggested that the protein concentration is above 2.5 mg/mL. Otherwise, increasing protein concentration is required before the experiment. The components (or storage buffer) of the sample to be labeled should meet the following requirements:

(1) Do not contain amino components, otherwise it will affect the coupling effect.

(2) A small amount of BSA will not affect the coupling effect. it is suggested to use PBS as storage buffer.

(3) If the sample contains substances that may interfere with the labeling, it is suggested to replace the buffer with PBS. The specific method is as below.

Add the sample to the ultrafiltration tube and add 200-150 µL PBS. Centrifuge at 12,000 g, 4°C, 10 min, and discard the filtrate. Then PBS was added again, and centrifuge at 12,000 g, 4°C, 10 min. After centrifugation, the inner core of the ultrafiltration tube is taken out, placed it in a clean outer tube, and centrifuge at 4,000 g, 4°C, 2 min to collect the sample.

List of components (or storage buffer) requirements of the sample to be labeled:

pH	**6.5-8.0**
Amine free buffer	MES, PBS, HEPES
Chelating agents (e. g. EDTA)	✓
Glycerol	< 50%
BSA	< 0.1%
Glycine	✗
Components containing amino	✗

Assay Procedure

Note: Please enlarge or reduce the volume of each component of the kit in the reaction system according to the actual sample volume.(For example like FlyLink™ AF 488 Labeling Kit)

The recommended optimal labeling sample size and labeling system for different specifications are as follows:

Size	Amount of Sample	Optimal Labeling System
3 ×100 μg	3×100 μg	3×50-3×75 μL
1 mg	1 mg	500-750 μL

The following operation steps are based on the 3×100 μg specification. For other specifications, please adjust the dosage accordingly. If you need to adjust the volume, please keep the sample size unchanged and change the volume of FlyLink™ AF 488 labeling solution, Activated FlyLink™ AF 488 solution and deionized water in the labeling system.

1. Add 15 μL FlyLink™ AF 488 labeling solution to the sample solution to be labeled and mix gently with a pipette.

2. Pipette 7.5 μL of Activated FlyLink™ AF 488 solution to the reaction solution in step 1, add deionized water to 150 μL (**Note: this volume is the labeled system**), mix gently, and let stand at 37°C in the dark for 1 h.

Note: Step 1/2 belong to the labeling steps.

3. Add an appropriate amount of PBS (fixed volume to about 500 μL) to the reaction solution in step 2, mix gently, and move the solution to the purification column. Centrifuge at 12,000 g at 4°C for 10 min.

4. Discard the filtrate and add an appropriate amount of PBS (fixed volume to about 500 μL) to the purification column. Centrifuge at 12,000 g for 10 min at 4°C.

5. Take out the purification column, put it upside down in a clean centrifuge tube, centrifuge at 4,000 g for 2 min at 4°C. The solution collected in the centrifuge tube is the coupling product.

Note: Step 3/4/5 are the purification steps. The conjugate can be stable at 4°C in the dark for more than one month. For long-term preservation, the conjugate should be packed in small aliquots, placed at -20°C in the dark and added the same volume of glycerol. Avoid repeated freezing and thawing.

Data Analysis

1. Calculation of Conjugates Concentration:

C (mg/mL) = {[A_{280}-(A_{max}×Cf)]÷1.4}×dilution factor

Where: C, the concentration of conjugates collected in the experiment; Dilution factor: Dilution factor in photometric measurement;

A_{280} and A_{max}: The absorbance at 280 nm and the absorbance at the maximum absorption wavelength (the maximum absorption wavelength of FlyLink™ AF 488 is 490 nm); Cf: The correction factor. The Cf value of FlyLink™ AF 488 is 0.1; 1.4: The extinction coefficient of IgG (mL/mg). The extinction coefficient of FlyLink™ AF 488 is 70,000.

2. Calculation of Degree of Labeling (DOL) of Conjugates:

DOL=(A_{max}×Mwt×dilution factor)÷(ε×C)

Where: A_{max}: The absorbance at the maximum absorption wavelength (the maximum absorption wavelength of FlyLink™ AF 488 is 490 nm); Dilution factor: Dilution factor in photometric measurement; C: The concentration of conjugates collected in the experiment; Mwt: The molecular weight of the sample to be labeled; ε: The extinction coefficient of fluorescent dyes, the extinction coefficient of FlyLink™ AF 488 is 70,000.

Note: The conjugate eluted by the column may be too concentrated for absorbance detection directly, so it needs to be diluted to appropriate concentration (Within the concentration detected by protein analyzer). The dilution multiple (i.e., dilution factor) needs to be estimated from the initial sample mass and the total volume of eluted conjugate. If undiluted, the dilution factor is 1.

FAQ

Q1: After concentration, the sample concentration is still below 2 mg/mL?

A1: If the sample concentration is still less than 2 mg/mL after concentration, the volume of the labeling system can be adjusted appropriately, but the final concentration should be greater than 1 mg/mL. The volume of the FlyLink™ AF 488 labeling solution in step 1 should be 10% of the volume of the labeling system. In step 2, deionized water may not be added and the volume of the Activated FlyLink™ 488 solution remains unchanged. You can also make appropriate adjustments based on your own experiments.

Q2: How to select the appropriate purification column for different molecular weight of the sample to be labeled?

A2: The purified column is suitable for samples with molecular weights ranging from 100 KD to 200 KD. If the samples with molecular weights higher than 200 KD or less than 100 KD, it should better be equipped with more suitable size of the purified column.

Q3: Is the molecular weight of the sample to be labeled similar to FlyLink™ AF 488?

A3: If the molecular weight of the sample is similar to FlyLink™ AF 488, after completing the labeling step corresponding to this instruction, add 30 µL 0.5 µM NH_4Cl and incubate at 37°C for 10 min to quench the free FlyLink™ AF 488 without the need for purification steps.

Q4: Relevant parameters of AbFluor™ series fluorescent dyes?

A4: See the following table.

Dye name	Maximum Excitation/ Emission Light (nm)	Extinction Coefficient (ε)	Correction Factor (Cf)	Molecular Weight (Mwt Da)
FlyLink™ 488	490/515	70,000	0.1	~834
FlyLink™ 555	555/565	150,000	0.08	~1077
FlyLink™ 594	593/614	115,000	0.08	~927
FlyLink™ 647	650/665	240,000	0.03	~1258
FlyLink™ 680	680/701	140,000	0.32	~1111

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.