

# CE method for Erythropoietin analysis according to Ph Eur.

This form describes the CE method for erythropoietin in solution according to the Ph. Eur. v6.0 (01/2008:1316) and should be repeatable.

## General remarks:

- Always use clean and dry vials
- Fill the 4 mL vials with 2.5 mL solution
- Use a new capillary



## Preparation of samples and buffers:

### Test solution:

- Dilute the EPO sample with demineralised water to a concentration of approximately 1 mg/mL.
- Desalt 0.25 mL of this solution through a microconcentrator cartridge with a cut-off of 10,000 Da.
- Add 0.2 mL water to this sample and desalt again.
- Add 0.2 mL water to this sample and desalt again.
- Add water to this sample to obtain a concentration of approximately 1 mg/mL.

### CZE buffer concentrate:

- Dissolve:
  - 0.584 g Sodium Chloride R
  - 1.792 g Tricine R
  - 0.820 Anhydrous sodium acetate R
- In 100 mL demineralised water. This will result in a solution containing 0.1M NaCl, 0.1M Tricine and 0.1M NaAc).

**1M putrescine:** dissolve 0.882 g putrescine R in 10 mL demineralised water.

### CZE Buffer:

- Dissolve 21.0 g Urea R in 25 mL demineralised water by warming in a water bath at 30°C.
- Add 5.0 mL CZE buffer concentrate to the urea solution.
- Add 125 µL 1M putrescine to this solution.
- Dilute this solution to 50.0 mL with demineralised water.
- Adjust the pH of this solution to 5.55 (T=30°C) with 1M acetic acid
- Filter this solution through a 0.45µm membrane filter.



**0.1M NaOH:** Dissolve 40.0 mg NaOH in 10 mL demineralised water and filter this solution through a 0.45µm membrane filter.

### Capillary:

- Material: bare fused silica capillary
- Internal diameter: 50  $\mu\text{m}$
- Effective length: 100 cm
- Capillary temperature: 35°C

### Coating procedure

Vial position	Solution
101	0.1M NaOH
102	CZE buffer
103	CZE buffer
105	Waste vial



Step	Inlet	Fluid	Outlet	Fluid	P@	P(mbar)	kV/ $\mu\text{A}$	kV/ $\mu\text{A}$	Dur(min)	Measure
1	101	0.1M NaOH	105	Waste	Inlet	1500	kV	0	60	No
2	102	CZE buffer	105	Waste	Inlet	1500	kV	0	60	No
3	102	CZE buffer	103	CZE buffer	Inlet	0	kV	20 kV	720	No

### Running method

Vial position	Solution
111	0.1M NaOH
112	Demin. water
113	CZE buffer
114	CZE buffer
115	CZE buffer
104	Waste



Step	Inlet	Fluid	Outlet	Fluid	P@	P(mbar)	kV/ $\mu\text{A}$	kV/ $\mu\text{A}$	Dur(min)	Measure
1	112	Water	104	waste	Inlet	1000	kV	0	10	No
2	111	0.1M NaOH	104	waste	Inlet	1000	kV	0	5	No
3	113	CZE buffer	104	waste	Inlet	1000	kV	0	10	No
4	Smpl	Sample	115	CZE buffer	Inlet	100	kV	0	0.2	No
5	114	CZE buffer	115	CZE buffer	Inlet	0	kV	19.3 kV	80	Yes
6	112	Water	104	waste	Inlet	1000	kV	0	5	No

Oven temperature: 35°C

Tray temperature: 10°C

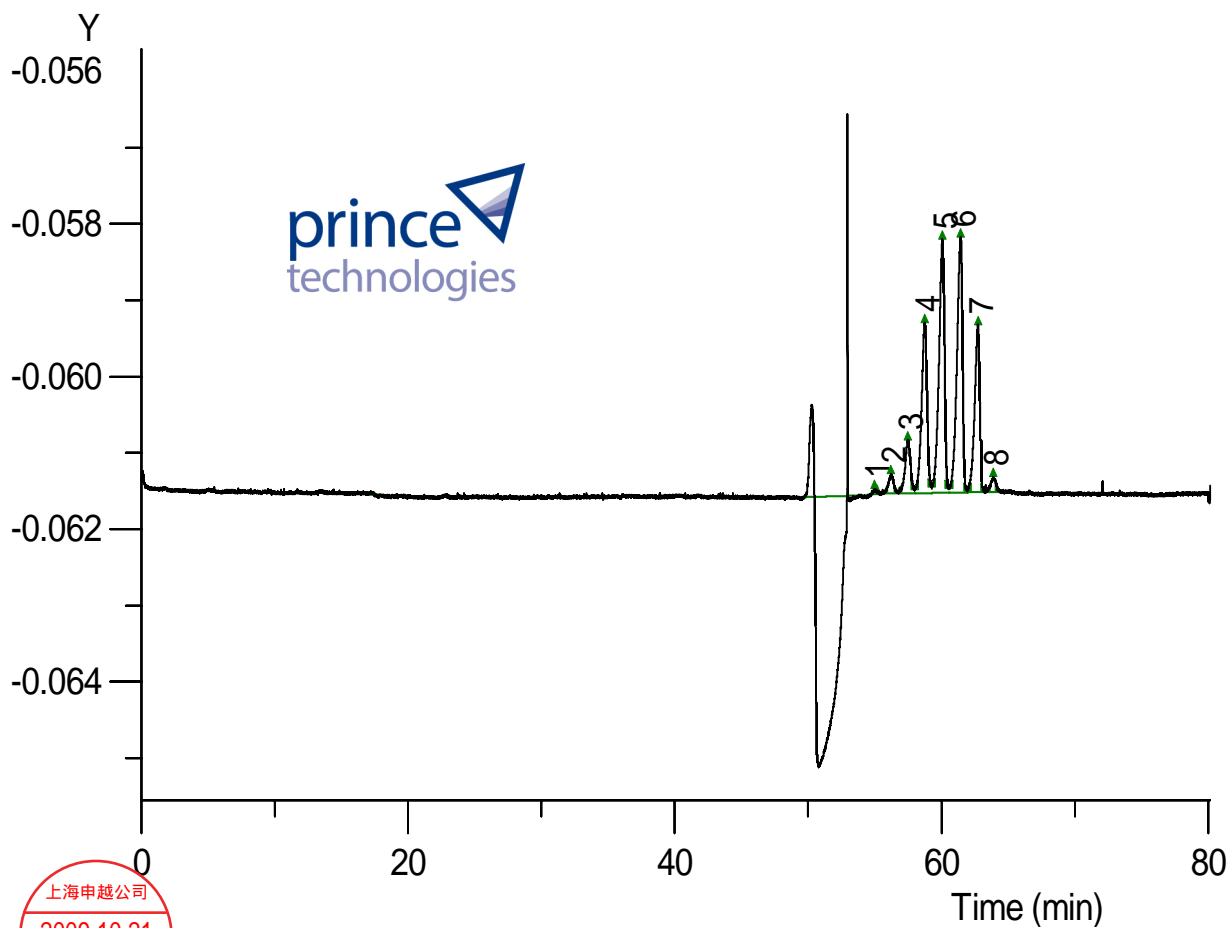
Ramps: begin and end 2 kV/s

Detection at 214 nm.

### Result

DAX 8.0 2009-8-10 11:15:22 sy

090807-epo-brp-03.da1 \*



上海申越公司  
2009.10.21

Component	Top (min)	Height (V)	Width (min)	Area (V.min)	Area Fract. %	Resolution
isoform1	55.002	6.652E-05	0.67500	1.9381E-05	0.333%	
isoform2	56.248	0.00025255	1.04333	0.00011557	1.99%	1.45
isoform3	57.498	0.00069751	1.25667	0.00033519	5.76%	1.09
isoform4	58.773	0.0022315	1.25167	0.0010774	18.5%	1.02
isoform5	60.102	0.003322	1.28167	0.0016119	27.7%	1.05
isoform6	61.457	0.0033439	1.33500	0.0016185	27.8%	1.04
isoform7	62.757	0.0021907	1.29833	0.00095824	16.5%	0.99
isoform8	63.920	0.00019174	0.94833	8.3945E-05	1.44%	1.04

reproducibility

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- 090807-epo-brp-03.da1
- 090807-epo-brp-04.da1

