SDS 电泳半干新技术

郭尧君　闻　弢*

（中国科学院生物物理研究所，北京 100101）

摘要　在 SDS 薄层凝胶水平电泳中应用半干技术缩短了电泳时间，提高了分辨率，简化了操作，免除经常大量配制电极缓冲液的麻烦．用滤纸条代替电极缓冲液和纸桥或代替凝胶条的新技术更方便．

关键词　SDS 电泳，半干技术，滤纸条

缓冲液是 SDS 电泳分离系统中很重要的介质．电极缓冲系统则影响电泳的时间和效果．过去使用约 500—2000ml 新鲜配制的电极缓冲液配以纸桥的方法既麻烦又浪费，而且电泳需要 2—4h[1-3]．80 年代末瑞典 pharmacia 公司[4]推出缓冲凝胶条．大大缩短了电泳时间，免除了经常配制大量电极缓冲液的麻烦．Seymour 等[5]在 1993 年国际电泳学术会上介绍了以滤纸作为电极缓冲液载体的新技术，既无需配制大量缓冲液或灌制胶条，又能取得良好的效果，而且更为方便．我们使用国产试剂和滤纸证实这一新技术的可行性和实用性．

1　材料与方法

1.1　SDS 梯度胶的准备

根据 SDS 聚丙烯酰胺小孔梯度胶的灌制方法[6]，灌制 9%—18%的梯度胶．凝胶缓冲液为 Tris/HCl pH8.8，胶厚度为 0.5mm.

1.2　电极缓冲液的配制

阳极缓冲液（pH6.4）：为 0.3mol/L Tris/乙酸，0.1% SDS，0.01% NaN_3 和 0.005% 溴酚蓝.

阴极缓冲液（pH7.1）：为 0.8mol/L 三羟甲基氨基甘氨酸（tricine），0.08mol/L Tris，0.1%SDS 和 0.01% NaN_3.

1.3　滤纸电极的准备

滤纸的宽度为 55mm，长度与胶长相同．不同型号的滤纸要使用不同的层数．八层新华 102 中速定性滤纸或四层 Whatman No.3 滤纸较合适．将准备好的滤纸以适量的缓冲液润湿，轻压赶去滤纸间气泡.

1.4　电泳

这一技术可在瑞典 pharmacia 公司 2117 Multiphor System 或其它合适的水平电泳仪上进行．先将胶水平放置于冷却板上，把润湿好的滤纸分别放于胶的阴阳两端．先将浸润了阴极缓冲液的阴极滤纸置于胶的阴极端，并使其距加样孔（或准备加样处）4mm，再放阳极滤纸使之与胶的阳极端搭接宽度为5mm．用弯头

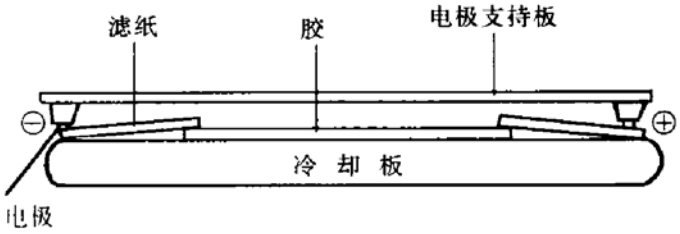

图 1　滤纸与电极放置图

表 1　SDS 凝胶电泳参数表

	电压/V	电流/mA	功率/W	时间/min
设置参数	600	40	16	80[1)]
起始状态	～400	40	～16	
终止状态	600	～20	～12	80

[1)]包括预电泳 10min，胶长 125mm，分离距离 80mm，冷却温度 10℃.

*中国科学技术大学生物系 89 级本科生.

收稿日期：1994-01-05，修回日期：1994-02-05

镊子先阴极后阳极轻轻压平滤纸，赶走气泡使胶面与滤纸接触良好．移动阴、阳电极，使其尽量平行并靠近滤纸外边缘，见图 1．预电泳后加样．电泳参数见表 1．

1.5 染色保存

电泳后进行考玛斯亮蓝染色和保存，方法见文献 [6]．

2 结果与讨论

2.1 电参数的变化

电泳过程中，电压不断上升，电流不断下降．由于电场和梯度胶的分子筛效应，样品带逐步被分离并被压缩变细．电压约在 15min 后达最大值．70—80min 后（包括预电泳），电流基本不再下降，指示剂前沿接近阳极，电泳结束．

早期 Görg 等[1]使用电极缓冲液配纸桥，进行 SDS 聚丙烯酰胺小孔梯度胶水平电泳时，以恒电流的方式进行，电泳时间一般在 2h 以上．而 Sinha 等[2]以及 Bjellqvist 等[3]的类似实验要 3—4h 才能完成．使用凝胶条和预制胶（ExcelGel SDS 胶：0.5mm × 125mm × 250mm）在参数设置为 600V，50mA，30W 时，也需 70min 左右[4]．由此可以看出，使用缓冲液润湿的滤纸条进行 SDS 梯度胶水平电泳，可以缩短电泳时间，操作方便，电泳效果良好，见图 2．

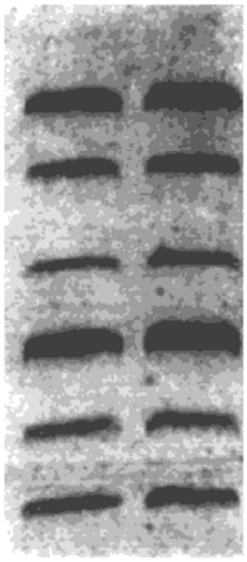

图 2 低分子量标准蛋白的 SDS 电泳

2.2 电极缓冲液与滤纸的使用

电极缓冲液的用量和滤纸的长度根据胶的尺寸决定．0.5mm × 125mm × 250mm 胶使用 20ml 缓冲液，滤纸长度与胶长相同．

所使用的滤纸要干净，操作过程中要避免玷污滤纸．先放阴极滤纸就是为避免阳极上的指示剂玷污阴极而影响指示剂前沿的观察．滤纸的润湿程度对电泳影响也很大．太湿会引起烧胶，太干或润湿不匀会影响滤纸与电极和凝胶的良好接触，使指示剂前沿弯曲倾斜甚至使电泳不能完成．滤纸在剪裁时要使其边缘平直整齐．使用八层新华滤纸或四层 Whatman No. 3 滤纸无明显差别．

2.3 预电泳

自制凝胶一般需进行预电泳，以避免凝胶中的杂质干扰电泳．10min 左右的预电泳较合适，时间太短起不到净胶作用；时间太长会使电解产物进入凝胶，电极缓冲液成分也会发生改变，都会影响最终的电泳结果．

参 考 文 献

1 Görg A, Postel W, Westermeier R *et al*. J Biochem Biophys Methods, 1980; 3: 273

2 Sinha P K, Praus M, Köttgen E *et al*. J Biochem Biophys Methods, 1990; 21: 173

3 Bjellqvist B, Ek K, Righetti P G *et al*. J Biochem Biophys Methods, 1982; 6: 317

4 Pharmacia LKB Biotech A B. Instruction Manual of ExcelGel™ precast gels and buffer strips for horizontal SDS electrophoresis. 1989; 3

5 Seymour C, Gronau S, Schickle H P. In: Solum N O ed. Program and Abstracts, ICES-ELPHO 93, Sandefjord, 1993; 125

6 郭尧君. 生物化学与生物物理进展，1991; 18 (1): 32

coefficient of variation (*CV*s) of two samples were 3.8% and 4.7%. Day-to-day coefficient of variation (*CV*s) was 7.0%. The correlation of immunoprecipitation method and electrophoretic procedure were cinsistent ($n=22$, $r=0.976$). Reference values for total LD and LD1 were 102.3±16.4U/L and 23.7±4.4U/L respectively. The ratio of LD1 to total LD was 23.1±3.9%. The advantages of immunoprecipitation method were: high specificity. good precision and linearity. easy operation. Immunoprecipitation method was very suitable for measuring LD1 and could adaptable to the automatic analyzer.

Key words isoenzyme 1 of lactate dehydrogenase (LD1). immunoprecipitation method. anti-M antibody

New Semi-dry Technique in SDS PAGE. Guo Yaojun. Wen Tao. (*Institute of Biophysics, Academia Sinica. Beijing* 100101). *Prog. Biochem. Biophys.* (China). 1994: **21** (3): 265

Shorter running time. higher resolution. simpler operation without preparation of large amount of electrode buffer is the superior character of SDS PAGE semi-dry technique. It is more convenient with buffer soaked filter strips instead of electrode buffer with filter bridge or buffer gel strips.

Key words semi-dry technique. SDS PAGE. filter strips

A New Assay for Kinase Activity: The Determination of Protein Kinase Using Capillary Electrophoresis. Wan Qian. Chen Changzheng. Li Boliang. Xia Qichang. (*Shanghai Institute of Biochemistry, Academia Sinica. Shanghai* 200031). *Prog. Biochem. Biophys.* (China). 1994: **21** (3): 267

A new assay of protein kinase A using capillary electrophoresis has been established. It is a universal and useful method for kinase activity. The method based on principle that substrate (kemptide) and product (phosphorylated kemptide) are easily seperated by capillary electrophoresis and the enzyme activity can be calculated on the integrated area. A continous sampling technique which can analyse more than 10 samples in one run has also been developed. The new method is easy to operate and its accuracy and sensitivity are higher than that of conventional isotopic method.

Key words protein kinase A activity. capillary electrophoresis. phosphorylation

The Variation of Content in *Tetrahymena pyriformis* During Ageing. Wang Bing. Liu Biansheng. Xing Yiyin. (*Hubei Geriatric Institute. Wuhan* 430071). *Prog. Biochem. Biophys.* (China). 1994: **21** (3): 268

Digital Image Analysis is a new DNA content measuring method in cell nucleus. During the senile process of *Tetrahymena pyriformis*. change of DNA content in nucleus could be measured by this method. According to law of Beer-Larmbert. cell nucleus in different growth period showed change of nuclear DNA content using level of nuclear integral optical density. The method possess quick measuring speed. well repetition. simple operation and good results. The results showed: when *Tetrahymena pyriformis* began the logarithmic growth phase. the nuclear DNA content reached peak gradually. When cell ageing gradually. the times of cell division as well as DNA content would be gradually decreased.

Key words *Tetrahymena pyriformis*. DNA content. digital image analysis. ageing