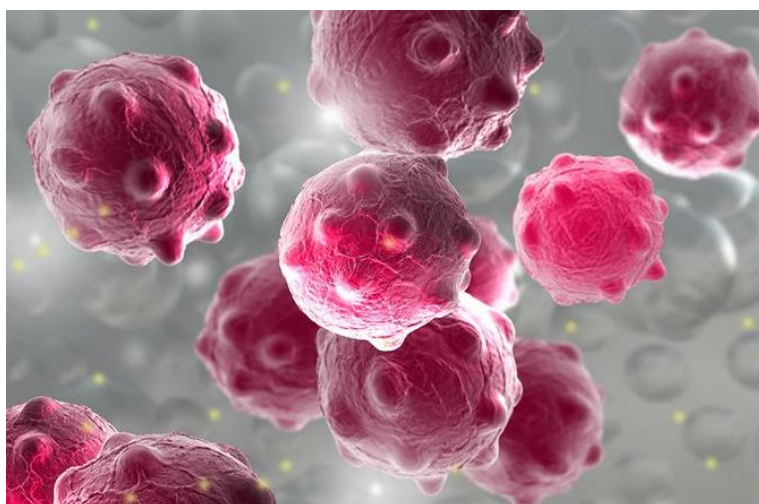


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第 130 期

东曹色谱产品在病毒关联药物和疫苗研发中的应用



HIGH QUALITY & EXPERT

第 130 期 东曹色谱产品在病毒关联药物和疫苗研发中的应用

病毒是一种个体微小，结构简单，只含一种核酸（DNA 或 RNA），必须在活细胞内寄生并以复制方式增殖的非细胞型生物。

病毒是一种非细胞生命形态，它由一个核酸长链和蛋白质外壳构成，病毒没有自己的代谢机构，没有酶系统。因此病毒离开了宿主细胞，就成了没有任何生命活动、也不能独立自我繁殖的化学物质。它的复制、转录和转译的能力都是在宿主细胞中进行，当它进入宿主细胞后，它就可以利用细胞中的物质和能量完成生命活动，按照它自己的核酸所包含的遗传信息产生和它一样的新一代病毒。

一、病毒和疫苗的特性

※ 疫苗

- 蛋白质作为表面抗原
- 天然蛋白、多糖或重组蛋白
- 类似于蛋白质分离的纯化策略
- 纯化用到的分离模式 SEC、IEC、HIC 和 AFC

※ 病毒

- 巨大的生物分子复合粒子
- 流感病毒：100-120nm；腺病毒：80nm；天花病毒：30nm
- 不同纯化策略对蛋白质的分离有不同的影响

二、疫苗用色谱填料

※ 尺寸排除色谱法（SEC）

- 病毒分离需要大孔径的填料

病毒尺寸：100nm----TOYOPEARL HW-65, Sepharose 6 FF

病毒尺寸：200nm---- TOYOPEARL HW-75, Sepharose 4 FF

- 填料的刚性对装柱更为重要

TOYOPEARL 比 Sepharose 基质具有更好的刚性和稳定性。

※ 离子交换色谱法 (IEC)

- 大孔径的填料: TOYOPEARL 650 系列
- 接枝型的聚甲基丙烯酸甲酯, 分离效果会更好, 如 GigaCap Q-650M, GigaCap S-650M, SuperQ-650M

※ 疏水层析 (HIC)

- 不同填料的疏水性有差异
- 疏水性较低的填料可能更有效分离病毒
- DNA 在 HIC 填料上的保留较差

※ 亲和层析 (AFC)

- 染料: TOYOPEARL AF-Blue-650M
- 肝素: TOYOPEARL AF-Heparin-HC-650M
- 其他蛋白质固定化 AFC 树脂

在这些关联药物的研发、评价、纯化精制中, TSKgel 液相色谱柱和 Toyopearl 层析填料产品有着相当多的应用实例。

TSKgel 色谱柱分析柱的应用实例

Year	Mode	TSKgel Column	Target,keyword	Title	Citation at web site
2020	SEC	TSKgel G5000PWXL	Coronavirus protein/ 冠状病毒蛋白	Characterization of the SARS-CoV-2 Spike in an Early Prefusion Conformation/早期融合前构象中 SARS-CoV-2 峰的特征	https://www.biorxiv.org/content/10.1101/2020.03.16.994152v1.full
2020	IEC	TSKgel DNA-STAT	PS-oligonucleotide / PS-寡聚核苷酸	Condition Study of Phosphorothioate Oligonucleotides Separation by High-performance Anion Exchange Chromatography /硫代磷酸寡聚核苷的阴离子高效液相色谱分析的条件研究	Poster presentation in Asia TIDES 2020
2019	SEC	TSKgel UP-SW2000	Oligonucleotide / 寡聚核苷酸	Analysis of oligonucleotide by SEC /寡聚核苷酸的分子尺寸排阻色谱分析	https://www.separations.us.tosohbioscience.com/HPLC_Columns/id-8694/TSKgel_UP-SW2000?invalid=1&Target=uJzmmWx7eqe3bbXcid9Eq/UAKSx5S2pFX8hW+KqJ6YMeIGVvJ+3p89zBvATw8mUQ7DWvS7VERS6O+kyfR/mkqQ==
2019	RPC IEC	TSKgel ODS-120H DNA-STAT	Oligonucleotide / 寡聚核苷酸	Examination of analytical conditions for separation of oligonucleotides for LC/MS /液相色谱进行寡聚核苷酸分析条件的检验	Poster presentation in HPLC Kyoto, 2019
2017	SEC	TSKgel G6000PWXL G5000PWXL	Conjugated vaccine / 结合疫苗	Determination of free polysaccharide in Vi glycoconjugate vaccine against typhoid fever /伤寒疫苗中游离多糖的测定	https://www.sciencedirect.com/science/article/pii/S0731708516312511?via%3Dihub
2017	SEC	TSKgel G5000PWXL	Virus-like particle / 类病毒颗粒 (VLP)	Quantification and characterization of virus-like particles by size-exclusion chromatography and nanoparticle tracking analysis / 通过尺寸排除色谱和纳米颗粒跟踪定性和定量分析类病毒颗粒	https://www.sciencedirect.com/science/article/pii/S0021967316317514
2016	SEC	TSKgel G3000PWXL	Vaccine/疫苗	Immunogenicity and protective efficacy of rotavirus VP8 fused to cholera toxin B subunit in a mouse model/轮状病毒	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5137547/

				毒 VP8 与霍乱毒素 B 亚单位融合在小鼠模型中的免疫原性和保护作用	
2016	SEC	TSKgel G6000PWXL	Flu virus(VP)/流感病毒	Size distribution analysis of influenza virus particles using size exclusion chromatography /流感病毒颗粒大小分布的分子尺寸排阻色谱分析	https://www.sciencedirect.com/science/article/pii/S0021967316311335
2015	SEC	TSKgel G5000PWXL G4000PWXL	Conjugated vaccine/ 结合疫苗	Development and characterization of Haemophilus influenzae type b conjugate vaccine prepared using different polysaccharide chain lengths /不同多糖链长流感嗜血杆菌 B 型偶联疫苗的研制与表征	https://www.sciencedirect.com/science/article/pii/S0264410X15004958?via%3Dihub
2014	SEC	TSKgel G5000PWXL-CP	Bacteriophage particle/噬菌体颗粒	Size exclusion-based purification and PCR-based quantitation of MS2 bacteriophage particles for environmental applications/用于环境应用的 MS2 噬菌体颗粒的储存排阻纯化与 PCR 定量	https://www.sciencedirect.com/science/article/pii/S0166093414004704?via%3Dihub
2013	SEC	TSKgel G5000PWXL-CP	Virus particle/病毒颗粒	A Gel Filtration -Based Method for the Purification of Infectious Rotavirus Particles for Environmental Research Applications /一种用于环境研究应用的感染性轮状病毒的凝胶过滤纯化方法	https://link.springer.com/article/10.1007/s12560-013-9122-4
2013	HIC	TSKgel Butyl-NPR	DNA vaccine/DNA 疫苗	Antibiotic-free production of a herpes simplex virus 2 DNA vaccine in a high yield cGMP process /无抗生素生产单纯疱疹病毒 2 型 DNA 疫苗的高产 cGMP 过程	https://www.tandfonline.com/doi/full/10.4161/hv.25048
2008	SEC	TSKgel G4000PWXL	Flu vaccine/流感疫苗	Development of physicochemical methods for analysis of pandemic influenza vaccine/大流行性流感疫苗理化分析方法的发展	https://journal.lib.uoguelph.ca/index.php/surg/article/view/420
2007	IEC	TSKgel DNA-NPR	Plasmid DNA/质粒 DNA	Separation of topological forms plasmid DNA by anion-exchange HPLC: Shifts in elution order of linear DNA/阴离子交换高效液相色谱法分离质粒 DNA 的拓扑结构	https://www.sciencedirect.com/science/article/pii/S1570023207002747
2007	SEC	TSKgel G5000PWXL	Conjugated vaccine / 结合疫苗	Quality and Immunogenicity of MenAfriVac, a Conjugate Vaccine Against Meningococcal Group A Disease/脑膜炎球菌 a 群疾病的结合疫苗 MenAfriVac 的质量和免疫原性	https://www.meningvax.org/files/PharmaPhase2PosterSept07.pdf

2007	SEC	TSKgel G5000PWXL	Flu vaccine /流感疫苗	A Retrospective Study on the Quality of Haemophilus influenzae type b Vaccines Used in the UK Between 1996 and 2004 / 对1996年至2004年英国使用的B型流感嗜血杆菌疫苗的质量进行回顾性研究	https://www.tandfonline.com/doi/abs/10.4161/hv.3.5.4352
2005	SEC	TSKgel G-DNA-PW G6000PWXL	Plasmid DNA/质粒DNA	High-speed chromatograph purification of plasmid DNA with a customized biporous hydrophobic adsorbent/用定制的双孔疏水吸附剂对质粒DNA进行高速色谱纯化	https://www.sciencedirect.com/science/article/abs/pii/S1369703X05002044
2003	SEC	TSKgel G5000PWXL G4000PWXL	Liposome/脂质体	Characterization of loaded liposomes by size exclusion chromatography/已包载脂质体的分子尺寸排阻色谱分析	https://www.sciencedirect.com/science/article/pii/S0165022X03000599?via%3Dihub

Toyopearl 层析填料的分离纯化应用实例

Year	Mode	TSKgel Column	Target,keyword	Title	Citation at web site
2019	HIC	PPG-600M	Flu virus/流感病毒	Hydrophobic-interaction chromatography for purification of influenza A and B virus / 疏水层析纯化甲型和乙型流感病毒	https://www.sciencedirect.com/science/article/pii/S1570023219301655?via%3Dihub
2019	IEC	TSKgel SuperQ-5PW	Nucleotides/核苷酸	Generic anion -exchange chromatography method for analytical and preparative separation of nucleotides in the development and manufacture of drug substances / 通用阴离子交换色谱法用于核酸药物的研发、生产中的分析	https://www.sciencedirect.com/science/article/pii/S0021967318315255?via%3Dihub
2018	IEC	TSKgel SuperQ-5PW	siRNA	Comprehensive evaluation of canonical versus Dicericercer-substrate siRNA in vitro and in vivo	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3285942/
2018	SEC	HW-65F	Virus-like particle (VLP) / 类病毒颗粒	Dengue virus-like particles mimic the antigenic properties of the infectious dengue virus envelope / 等革类病毒颗粒模拟感染性登革病毒包膜的抗原特性	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5879749/

2017	HIC	Butyl-650M	BBC vaccine/BBC 疫苗	Multi-subunit BCG booster vaccine GamTBvac: Assessment of immunogenicity and protective efficacy in murine and guinea pig TB models/多亚单位卡介苗增强疫苗 GamTBvac: 小鼠和豚鼠结核病模型免疫原性和保护效果的评估	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5409163/
2016	HIC	Butyl-650M	Malaria vaccine/疟疾疫苗	Production, Quality Control, Stability and Pharmacotoxicity of a Malaria Vaccine Comprising Three Highly Similar PfAMA1 Protein Molecules to Overcome Antigenic Variation/由三种高度相似的 PfAMA1 蛋白分子组成 (克服抗原变异) 的疟疾疫苗的生产、质量控制、稳定性和药物毒性	https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0164053
2016	IEC	NH ₂ -750F GigaCap Q-650M	Flu virus/流感病毒	Mono- and polyprotic buffer systems in anion- exchange chromatography of influenza virus particles /流感病毒颗粒阴离子交换层析中单、多质子缓冲体系的研究	https://www.sciencedirect.com/science/article/pii/S0021967316304708?via%3Dihub
2015	AFC	AF-Heparin 650M	Pertussis vaccine /百日咳疫苗	Single Amino Acid Polymorphisms of Pertussis Toxin Subunit S2 (PtxB) Affect Protein Function /百日咳毒素亚基 S2(PtxB)单氨基酸多态性影响蛋白功能	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4573519/
2013	IEC	DEAE-650M	HBV-VLP/HBV 类病毒颗粒	Purification of hepatitis B surface antigen virus-like particles from recombinant Pichia pastoris and in vivo analysis of their immunogenic properties /从重组毕赤酵母中纯化乙型肝炎表面抗原样病毒颗粒, 并在体内分析其免疫原性	https://www.sciencedirect.com/science/article/pii/S1570023213005126?via%3Dihub
2012	HIC	Phenyl-600M	HBV vaccine/乙肝疫苗	Novel membrane extraction procedure for the purification of hepatitis B surface antigen from Pichia pastoris/新型膜提取法从毕赤酵母中分离纯化乙型肝炎表面抗原	https://www.sciencedirect.com/science/article/pii/S1570023212002115?via%3Dihub
2012	IEC	DEAE-650M	Anti-viral component/ 抗病毒成分	Isolation of antiviral components against Influenza from food in Yamagata (report Japanese) /从山形县食物中分离抗流感病毒成分	http://www.eiken.yamagata.yamagata.jp/pdfshohou/shohou45.pdf

2009	HIC (HAC)	Phenyl-650M (Ca++Pure-HA)	Botlinum vaccine/肉毒疫苗	Bivalent recombinant vaccine for botlinum neurotoxin types A and B based on a polypeptide comprising their effector and translocation domains that is protective against the predominant A and B subtypes / 基于包含其效应域和易位域的多肽肉毒杆菌神经毒素 A 型和 B 型的双价重组疫苗	https://www.ncbi.nlm.nih.gov/pubmed/19398544
2006	IEC	DEAE-650M	HBV vaccine/乙肝疫苗	Expression in and purification of Hepatitis B surface antigen (S-protein) from methylotrophic yeast Pichia pastoris/乙型肝炎表面抗原 (S-蛋白) 的表达及纯化	https://www.sciencedirect.com/science/article/pii/S107599640600045X?via%3Dihub
2004	IEC	DEAE-650M	Adenovirus/腺病毒	Development and optimization of an adenovirus production process/腺病毒生产工艺的开发和优化	https://onlinelibrary.wiley.com/doi/10.1002/jgm.503
2002	IEC SEC	SP-650M,HW-65S	Botlinum vaccine/肉毒疫苗	Mucosal immunisation with Clostridium botulinum type C 16 S toxoid and its non-toxic component / C16 S-型肉毒杆菌及其无毒成分的粘膜免疫	https://www.microbiologyresearch.org/content/journal/jmm/10.1099/0022-1317-51-10-813

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咨询电话: 010-59812370/1/2/3

公司官网: www.prep-hplc.com

邮箱: sales@prep-hplc.com

微信公众号: 北京慧德易