

Tech Tip 0002 - Botanical Reference Material Product Grades



Botanical Reference Materials (BRMs) are well characterized materials sampled from specific plants and plant parts. ChromaDex offers a product line of Botanical Reference Materials (BRM) to provide users with the tools necessary to better evaluate botanical raw materials. BRMs are typically freeze-dried and ground plant material. Their Certificate of Analysis show an HP-TLC test method, profile image, and may also include macro and microscopic images.

ChromaDex offers a wide range of BRMs grades with varying degrees of characterization. The available grades of Botanical Reference Materials are:

Vouchered Botanical Reference Materials (VBRMs)

VBRMs are highly characterized Botanical Reference Materials. The Certificate of Analysis for ChromaDex VBRMs includes macroscopic and microscopic images with HP-TLC fingerprints to confirm identity of the material. In addition, a voucher is collected at the time of harvest and the plant is visually identified. ChromaDex retains access to the full vouchered specimen that can be used for authentication at a later date. Upon request, an image of the voucher and/or full botanical authentication at an additional cost can be supplied.

Botanical Reference Materials (BRMs)

BRMs are well characterized Botanical Reference Materials. The Certificate of Analysis for ChromaDex BRMs includes both macroscopic and microscopic images with HP-TLC fingerprints to confirm the identity of the material.

Reagent Grade Botanical Reference Materials (RBRMs)

RBRMs are Botanical Reference Materials characterized only by HP-TLC fingerprint analysis, detailed on their Certificates of Analysis.

Extract Botanical Reference Materials (XBRMs)

XBRMs are powdered extracts of Botanical Reference Materials from a specific plant biomass. The Certificate of Analysis for ChromaDex[®] XBRMs includes HP-TLC fingerprints and quantitative HPLC/GC analysis of major chemical markers. As the extraction process cannot be guaranteed, they are not a substitute for actual Botanical Reference Materials. XBRMs should be used along with Reference Standards to provide robust analytical results.

American Herbal Pharmacopoeia (AHP-BRMs)

In partnership with the American Herbal Pharmacopoeia, ChromaDex offers several botanical reference materials as AHP verified. All AHP grade reference materials are VBRM grade reference materials that have been independently verified by the American Herbal Pharmacopoeia and will bear the AHP Verified logo. The certificate of analysis will also bear the AHP verified statement. This offers an additional level of traceability.

Tech Tip 0002 - Botanical Reference Material Product Grades

A full breakdown of the characterization details for each grade is outlined in the table below:

Grade	Plant Information	HP-TLC Fingerprint Images	Macroscopic Images	Microscopic Images	Botanical Voucher Images	HPLC/GC Analysis
Vouchered Botanical Reference Materials (VBRMs)	✓	✓	✓	✓	✓	
Botanical Reference Materials (BRMs)	✓	✓	✓	✓		
Reagent Grade Botanical Reference Materials (RBRMs)	✓	✓	✓			
Extract Botanical Reference Materials (XBRMs) [†]	✓	✓	✓			✓

The variety of Botanical Reference Material grades ChromaDex supplies offers versatility across a unique area of end users. Listed below is an overview of some typical uses and the appropriate Botanical Reference Materials for each.

Grade	Identity Verification	Raw Material Comparison	Thin Layer Chromatography (TLC)	Microscopy	Basic Research	Early Stage Method Development/validation	HPLC
Vouchered Botanical Reference Materials (VBRMs)	✓	✓	✓	✓	✓	✓	
Botanical Reference Materials (BRMs)	✓	✓	✓	✓	✓	✓	
Reagent Grade Botanical Reference Materials (RBRMs)					✓	✓	
Extract Botanical Reference Materials (XBRMs) [†]			✓*		✓	✓	✓*

*Applicable for TLC/HPLC analysis, but should not be used to establish botanical identity.

Tech Tip 0002 - Botanical Reference Material Product Grades

Example: VBRM Certificate of Analysis

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PRODUCT: Cucurbita pepo (Pumpkin) Seed
PART NUMBER: 0001332
REFERENCE TYPE: Botanic Reference Material (VBRM)*
LOT NUMBER: 0001332-0117
COMMON NAME: Pumpkin
LATIN NAME: Cucurbita pepo L. (Cucurbitaceae)
PLANT PART: Seed (ground material)
SAMPLE NUMBER: CDMX-0001332
REPORT NUMBER: CDXA-0684-203-00
DATE OF SAMPLE: 02/18/2021
DATE OF REPORT: 02/17/2021

*Note: Voucher (V) BRM is a voucher specimen and has not been established by microscopy techniques.
*Visible color voucher copy may be purchased by contacting the ChromaDex sales office.



ANALYTICAL RESULTS

TEST	METHOD	RESULT
Appearance	Microscopy	(1) Whole seeds with flat, convex, constricted at one end forming a short, blunt projection, up to 25 mm long and 12.5 mm wide; seeds compressible, white to pale beige, smooth or with irregular warts. (2) embryo pale greenish-yellow, (3) inner surface of the testa brownish-white, (4) margin of the testa is raised along the longitudinal groove running throughout the length of the seed. (5) Ground light brown powder.
Anatomy/Morphology	Microscopy (modified cleared hydrate procedure)	(1) Endo-spermatophyte and epidermal cells of the cotyledon. (2) 200X fragment of endosperm in surface view. Confirms.
HP-TLC	See HP-TLC conditions	

STORAGE CONDITIONS: 20-30°C. Dry storage area, frost-free, Vapour-free
SHIPPING: 02/20/2021 under the above conditions
EXPIRATION DATE: 12/31/2024 under the above conditions

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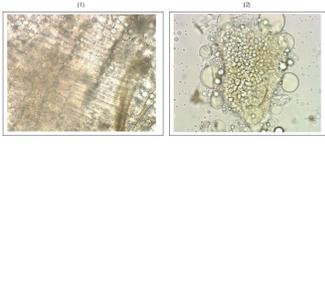
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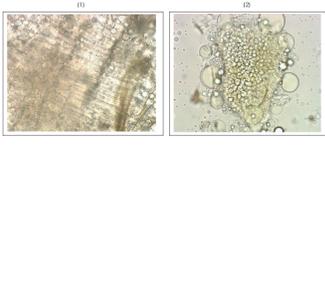


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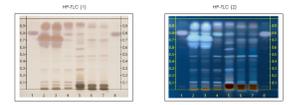
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HP-TLC CONDITIONS

STATIONARY PHASE: Silica gel 60, HP-TLC plates
SAMPLE PREPARATION: 0.3 g + 3 mL Methanol, sonicate/heat at 50°C for 30 min.
MOBILE PHASE: Chloroform: Methanol: Water (7:3:0)

DETECTION: (1) UV 254 nm, 100°C 2 min, V5
(2) 10% Sulfuric Acid, 100°C 2 min, Moken



HP-TLC LANE APPLICATIONS

Lot#	ID	Lot#	ID
1	Biotinylated Biotinylated (3 µL)	5	N/A
2	Cucurbita moschata seed (2 µL)	6	Cucurbita moschata leaf (4 µL)
3	Cucurbita pepo seed (4 µL)	7	Cucurbita pepo leaf (4 µL)
4	Cucurbita pepo (CDMP-1200) (7.4 µL)	8	β-Diosgenin Standard (3 µL)

REVISION HISTORY

Revision History	Date of Revision	Document/Change
00	03/17/2021	New report

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Example: BRM Certificate of Analysis

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PRODUCT: Rheum officinale/polygonum (Chinese Rhubarb) Root
PART NUMBER: 0003077
REFERENCE TYPE: Botanic Reference Material (BRM)*
LOT NUMBER: 0003077-0194
COMMON NAME: Chinese Rhubarb
LATIN NAME: Rheum officinale/polygonum L. var. (Polygonaceae)
PLANT PART: Root
SAMPLE NUMBER: CDXA-16-001725
REPORT NUMBER: CDXA-0680-47-01
DATE OF SAMPLE: 04/01/2014
DATE OF RE-EVALUATION: 03/09/2021
DATE OF REPORT: 03/23/2021

*Note: Botanic Reference Material (BRM) is not a voucher specimen.

ANALYTICAL RESULTS

TEST	METHOD	RESULT
Appearance	Microscopy	(1) Whole root: Rheum officinale rhizome of root (2) Rhizome: light brown powder (3) Rhizome: Rhubarb rhizome associated with pectin-rich and a mucilaginous in longitudinal longitudinal section. (20X)
Anatomy/Morphology	Microscopy	(2) Cellular analysis of calcium oxalate (20X) confirms.
HP-TLC	See HP-TLC conditions	

STORAGE CONDITIONS: 20-30°C. Dry storage area, frost-free, Vapour-free
SHIPPING: 02/20/2021 under the above conditions
EXPIRATION DATE: 12/31/2024 under the above conditions

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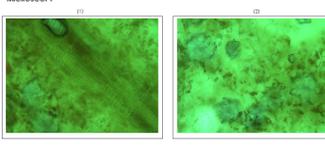
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MICROSCOPY



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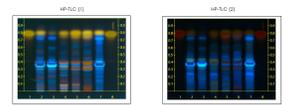
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HP-TLC CONDITIONS

STATIONARY PHASE: Silica gel 60, HP-TLC plates
SAMPLE PREPARATION: 0.3 g + 3 mL Methanol, sonicate/heat at 50°C for 30 min.
MOBILE PHASE: Ethyl acetate: Methanol: Water (10:1:30) (1) UV 254 nm (2) Natural Product + Polyethylene Glycol, UV 366 nm

DETECTION: (1) UV 254 nm (2) Natural Product + Polyethylene Glycol, UV 366 nm



HP-TLC LANE APPLICATIONS

Lot#	ID	Lot#	ID
1	Enodiol (3 µL)	5	Rheum officinale/polygonum; CDXA-16-001725 (2 µL)
2	Rheum officinale/polygonum (2 µL)	6	Rheum officinale/polygonum (2 µL)
3	Rheum officinale/polygonum (2 µL)	7	Rheum officinale/polygonum (2 µL)
4	N/A	8	Enodiol (3 µL)

REVISION HISTORY

Revision History	Date of Revision	Document/Change
00	04/07/2016	New report
01	03/07/2021	Material passed re-evaluation by HP-TLC, updated detection, mobile phase, and HP-TLC conditions, plates, and lane applications. A3300 image of ground material.

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Example: RGRM Certificate of Analysis

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PRODUCT: Ligusticum officinale (Gaugelie) Root
PART NUMBER: 0003186
REFERENCE TYPE: Botanic Reference Material (RGRM)*
LOT NUMBER: 0003186-407
COMMON NAME: Longie
LATIN NAME: Ligusticum officinale W. Koch (Apiaceae)
PLANT PART: Root
SAMPLE NUMBER: CDXA-09-3920
REPORT NUMBER: CDXA-RGRM14-002
DATE OF SAMPLE: 01/04/2011
DATE OF RE-EVALUATION: 01/29/2016 (1) 01/07/2021 (2)*
DATE OF REPORT: 01/29/2021

*Note: Reagent Grade (RGRM) is not a voucher specimen and has not been established by microscopy techniques.

ANALYTICAL RESULTS

TEST	METHOD	RESULT
Appearance	Microscopy	(1) Whole root: brown beige cut root (2) Root: tan colored, porous
HP-TLC	See HP-TLC conditions	

STORAGE CONDITIONS: 20-30°C. Dry storage area, frost-free, Vapour-free
SHIPPING: 02/20/2021 under the above conditions
EXPIRATION DATE: 12/31/2024 under the above conditions

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MACROSCOPY



HP-TLC CONDITIONS

STATIONARY PHASE: Silica gel 60, HP-TLC plates
SAMPLE PREPARATION: 0.3 g + 3 mL methanol, sonicate/heat at 50°C for 30 min.
MOBILE PHASE: Toluene: Ethyl Acetate: Formic Acid (9:1:0.1)
DETECTION: (1) 10% Sulfuric acid, 100°C for 2 min + 366 nm (2) 10% Sulfuric acid, 100°C for 2 min + 366 nm

HP-TLC LANE APPLICATIONS

Lot#	ID	Lot#	ID
1	Ligusticum officinale (RGRM) (2 µL)	5	Ligusticum officinale (RGRM) (2 µL)
2	Ligusticum officinale (RGRM) (2 µL)	6	Ligusticum officinale (RGRM) (2 µL)
3	Ligusticum officinale (RGRM) (2 µL)	7	Ligusticum officinale (RGRM) (2 µL)
4	Ligusticum officinale (RGRM) (2 µL)	8	β-Diosgenin (3 µL)

REVISION HISTORY

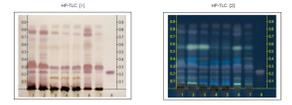
Revision History	Date of Revision	Document/Change
00	05/05/2011	New report
01	01/28/2016	Material re-evaluation, updated detection, general template update, updated detection, updated TLC plates, added revision history
02	01/29/2021	Material passed second re-evaluation by HP-TLC, updated detection, mobile phase, HP-TLC method conditions, plates, and lane applications.

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HP-TLC PLATES



HP-TLC LANE APPLICATIONS

Lot#	ID	Lot#	ID
1	Ligusticum officinale (RGRM) (2 µL)	5	Ligusticum officinale (RGRM) (2 µL)
2	Ligusticum officinale (RGRM) (2 µL)	6	Ligusticum officinale (RGRM) (2 µL)
3	Ligusticum officinale (RGRM) (2 µL)	7	Ligusticum officinale (RGRM) (2 µL)
4	Ligusticum officinale (RGRM) (2 µL)	8	β-Diosgenin (3 µL)

REVISION HISTORY

Revision History	Date of Revision	Document/Change
00	05/05/2011	New report
01	01/28/2016	Material re-evaluation, updated detection, general template update, updated detection, updated TLC plates, added revision history
02	01/29/2021	Material passed second re-evaluation by HP-TLC, updated detection, mobile phase, HP-TLC method conditions, plates, and lane applications.

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Tech Tip 0002 - Botanical Reference Material Product Grades

Example: XRM Certificate of Analysis

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PRODUCT	Coffea arabica (Green coffee) Bean
PART NUMBER	00031345
REFERENCE TYPE	Extract Reference Material (XRM)
LOT NUMBER	00031345-034
COMMON NAME	Green coffee
LATIN NAME	Coffea Arabica L. [Rubiaceae]
PLANT PART	Bean
CDXP NUMBER	CDXA-12-7446
REPORT NUMBER	CDXA-XRM-063-02
DATE OF SAMPLE	01/31/2013
DATE OF RE-EVALUATION	04/07/2017 [1]: 05/16/2019 [2=]
DATE OF REPORT	06/11/2019

ANALYTICAL RESULTS

TEST	METHOD	RESULT
Appearance	Macroscopy	Yellow/beige powder
HPLC	See below	Confirms
HP-TLC	CDXA-TLCM-116-01	Confirms

STORAGE CONDITIONS

STORAGE: 20-30 °C; Dry storage area; Insect free; Valiate free
 EXPIRATION DATE: 05/2024 under the above conditions

MACROSCOPY



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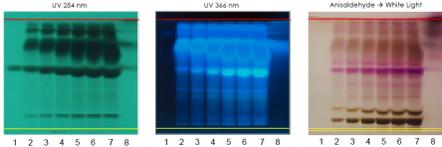
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HP-TLC CONDITIONS

STATIONARY PHASE: Silica gel 60, F₂₅₄, 10 x 10 cm HP-TLC plates
SAMPLE PREPARATION: 0.5 g + 5 mL water, sonicated in hot water bath for ~10 minutes and filtered through a 0.45 µm nylon syringe filter.
MOBILE PHASE: Ethyl acetate/ formic acid/ acetic acid/ MIB-Q water (100/1.1/1.1/2.6)
CHAMBER TEMPERATURE: Ambient
DETECTION: (1) UV 254 nm (2) UV 366 nm (3) Anisaldehyde → White Light

HP-TLC PLATES



YELLOW/WHI = SAMPLE ORIGIN
 RED LINE = SOLVENT FRONT @ 70 mm

Lane	ID	HP-TLC LANE APPLICATIONS	ID
1	Caffeine	5	Coffea arabica XRM
2	Coffea arabica XRM	6	Coffea arabica XRM
3	Coffea arabica XRM	7	Coffea arabica XRM
4	Coffea arabica XRM	8	Chlorogenic acid

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REVISION HISTORY

Revision History	Date of Revision	Document/Changes
00	02/04/2013	New report
01	04/24/2017	Passed first re-evaluation; updated expiration date; updated HPLC conditions, results and chromatograms; general template update
02	06/11/2019	Passed second re-evaluation by HP-TLC; Updated HP-TLC conditions and plates, template, and expiration date.

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HPLC RESULTS

ANALYTE	METHOD	RESULT
5-CQA	99-1-CD-8.0-000194	5.52%
Chlorogenic Acid	*Chlorogenic Acids by HPLC*	23.5%
4-CQA		5.28%
5-FQA		5.23%
3,4-Di CQA		2.65%
3,5-Di CQA		2.05%
4,5-Di CQA		3.32%
Related compounds		1.60%

ND = Not Detected

HPLC CONDITIONS

INSTRUMENT: Agilent 1100 HPLC UV-VIS DETECTOR (VWD)
COLUMN: Phenomenex Kinetex C18 100 x 4.6 mm, 5 µm particle size
MOBILE PHASE: A = 0.1% TFA (aq), B = Acetonitrile
 Time (min) SA SB
 0 95 5
 10.0 80 20
 12.0 80 20
 15.0 95 5
 15.1 5 95
 16.5 5 95
 16.6 95 5
 19.0 95 5

COLUMN TEMP.: 25 °C
FLOW RATE: 1.5 mL/minute
INJECTION VOL.: 3 µL
DETECTION: 330 nm

SAMPLE PREPARATION

Approximately 250 mg material was weighed into a 50 mL volumetric flask. 40 mL of 2% formic acid in 10% acetonitrile (aq.) was added and the solution was vortexed and sonicated for 10 min. The solution was equilibrated to room temperature, diluted to volume with MIB-Q water, vortexed and filtered through a 0.45 µm PTFE filter into an HPLC vial for analysis.

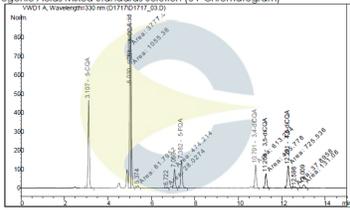
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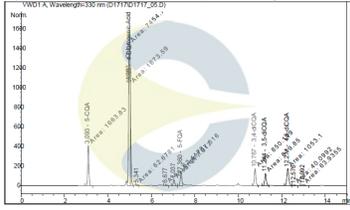
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HPLC CHROMATOGRAMS

Chlorogenic Acids Mixed Standards Solution (UV Chromatogram)



Coffee spp. (Green Coffee) Been CDXA-12-7446 (UV Chromatogram)



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